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Surgical Aspects of the Sympathetic Nervous System in the Work of René Leriche

By Wilder Penfield

AMONG medical men in general the name of Leriche is familiar because of the operation of periarterial sympathectomy which he fathered. His reputation has thus been, to a certain extent, dependent on the success the operation has achieved in the hands of other surgeons. The indications for the operation have often been ignored and the resultant failures misunderstood. A critical consideration of the field of his work may not be amiss at this time particularly as he has pointed out the important rôle of the sympathetic as seen in various branches of surgery.

It was in the hope of learning how Leriche himself would explain the evident inconsistencies between our theory of sympathetic function and the results of sympathectomy that I visited Lyon in the summer of 1924. I also hoped to discover a logical constructive point of view of the surgery of the sympathetic and in this I was not disappointed.

Rene Leriche has been "Professeur Agrégé" in the Surgical Faculty of the University of Lyon since 1910. He has been the energetic editor of the *Lyon chirurgical* and a prolific writer. Never having had a hospital service of of his own, the opportunity to begin his work on the arteries would never have presented itself, he said, had it not been for the war. Now, at the age of forty-four, he has just accepted the Chair of Surgery at the University of Strasbourg.

Dr. Leriche is short and powerfully built. He has a large head made to appear larger by rebellious curly hair. When in his study he frequently interrupts his talk to go to one of the many cupboards which line the room to take out a journal or book on anatomy. In this he quickly turns to the desired reference and asks his hearer to read a few lines as though he wished to prove his point. He is mentally a very strong visual, as he frequently illustrates his points with diagrams. It is apparent that he reads surgical literature consistently and the leading American surgical journals were on a stand beside the desk. The literature that he quoted and the books he referred to were surgical or anatomical except for the physiology of Claude Bernard. He impressed me as being frank and critical in matters of science and not unnecessarily egotistical.

The two uninterrupted talks we had in his office were the most concentrated and profitable interviews I have ever had. In the first, which lasted two hours, he outlined in a methodical way

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what he has contributed that was new in neurological surgery, and it made one realize more than ever that in the future the most stimulating and by far the largest part of the field of this specialty will lie outside the treatment of intracranial neoplasms. In the second talk, which began at two p.m., he answered a list of questions which I had prepared. He did this so fully that I left his office at six o'clock filled with enthusiasm because of the possibilities for work on the sympathetic nervous system.

PERIARTERIAL SYMPATHECTOMY

Peripheral, or periarterial sympathectomy, has brought to Leriche his fame. He was at some pains to explain that this operation was not done by Jaboulay, late Professor at Lyon, who had stretched sympathetic nerves but had never observed the primary arterial spasm resulting from removal.

From the femoral or brachial artery he removes[1] 6 to 10 cm. of adventitia. He uses very fine forceps, mosquito or Halsted artery clamps and a pointed scalpel, not the demountable variety. To start the separation he picks up the adventitia and stretches it away from the muscular wall. He then cuts through the adventitia and allows the gaping incision to fall back on the artery. When he reaches a branch, he incises the adventitia about its origin and leaves the branch intact unless it be small, when he cuts it between ligatures. In his two cases of sympathectomy of the internal iliac he denuded only as much of the artery as could be reached with safety, which was a few centimeters. The desired result was obtained.

First and last, Leriche emphasized the fact that there were theoretical inconsistencies he could not explain; that he observed and reported the facts as he had found them in something over thirty cases. Those who got different results, he said, either performed a different operation or were faulty observers. One objection, often cited, to sympathectomy is the existence of a certain number of non-medullated fibers which join the vessel from peripheral nerves below the site of the sympathectomy. The existence of these fibres no one can deny. Nevertheless, it is the experimental physiological and clinical results that must influence one's judgment finally.

The immediate result of periarterial sympathectomy is a primary stage of local contraction of the denuded artery which lasts from five to eight hours. This is followed by a secondary stage of peripheral active dilatation, which is associated with the return of an absent pulse, increased pressure compared with that of the opposite side and an increase of several degrees of temperature. This change sometimes is reported by the patient to come on suddenly. These two stages are invariable, whether the case be abnormal or normal, and if not

[1] As a surgeon he follows the French manner of operating, with one assistant, a self-retaining retractor, and no nurse. All instruments are lifted by an orderly direct from sterilizer to sterile table and all sheets come direct from drums. Thanks to the great simplicity, there was practically no break in aseptic technic. He thinks American surgery, of which he has seen a good deal, is a little too complicated. He worked deliberately and with great care, saying that he considered speed the enemy of good surgery.

observed the conclusion should be that the operation has not been performed, just as failure of pupillary change (he said) indicates that the pneumogastric has been removed in reported excision of the cervical sympathetic.

He does not know whether this second stage is accompanied by local dilatation of the denuded artery. He calls this peripheral dilatation *active* because Claude Bernard showed that the dilatation following sympathetic section in the neck must be called active rather than passive. Bernard gave as a reason for this the increase of heat observed, which would not be caused by a simple stasis.[2]

But that peripheral dilatation, or at least an increased circulation, can be caused by simple removal of the periarterial nerves is best proved by the following cases (two, I think). In these he removed an *obliterated* portion of the artery passing to a limb, without doing anything to the functioning vessels. Such an obliterated artery has been well called by Babinski a sympathetic nerve. In both cases the previously existing ischemia and so-called trophic changes disappeared almost immediately. The vasodilatation in these cases ceased in about three weeks just as after a sympathectomy.

In his hands success has followed operation in a number of conditions. Among others are the following:

1. Trophic and post-traumatic ulcerations. This does not include the ulcers caused by venous varicosities nor those due to general sclerotic destruction of the arterial supply to the limb.

2. Raynaud's Disease. This is not to be taken to mean thromboangeitis obliterans, an example of which has not been seen in the Lyon clinic.

3. Causalgia, as discussed below.

4. Various edemas and certain skin diseases.

[2] It would seem that, in the case of sympathectomy, a passive relaxation of the arteries in spasm would produce all the observed phenomena without stasis and without an active dilatation, but this is a matter of names.

CAUSALGIA

Leriche provides a new method of attack on the baffling problem of causalgia. He believes it is essentially a vasomotor phenomenon and strongly opposes the amputations and reamputations so often performed.

Causalgia, he says, may appear after (1) a slight trauma without evident vascular or nervous injury, (2) superficial lesions of the wall of an artery or vein, (3) obliteration of a vein or artery, (4) lesion of a nerve (almost always a nerve with an arteria propria as the median or sciatic). Assuming the pain to be of vasomotor origin he performed periarterial sympathectomies for treatment, beginning in 1919. At the International Congress of Surgery in London in 1923 he reported the results in nine such cases. Five of these results were good (return to normal with ability to work again). In two cases improvement was noted, and in two cases the operation failed. He recommends that the sympathectomy be done a good way above the site of injury, e.g., on the humeral artery for lesions of the hand and on the brachial or subclavian for lesions of the elbow. He says that it is too soon yet to speak of the ultimate results, but that if the duration of the

trouble has been too long and the pain has spread up the limb, the operation may come too late.

Leriche has made interesting observations on the pathology of causalgia. In cases in which he has sectioned the posterior roots for this pain, he has three times observed the following curious condition. After doing a laminectomy he saw numerous dilated vessels in the meninges on the side of the lumbar cord and conus toward the affected leg. He attributes the pains to the presence of these vessels which press upon nerve roots. In one case the vessels were bilateral and in this way he explains the spread of pain to the opposite side. He also observed some sort of deposit on the arachnoid, which he termed arachnoiditis, in the corresponding area.

Leriche is an opportunist in many ways. In casting about for a physiological explanation of the above phenomena he hit upon the sinu-vertebral nerve described by Luschka seventy years ago and allowed to rest in peace since that time. Luschka suggested that the nerve probably had to do with the bony pains and aches of the back. A sinu-vertebral nerve is formed near each spinal ganglion by a spinal and a sympathetic branch. It then enters the vertebral canal and ramifies for the most part on the vessels of the spinal roots, spinal cord and pia mater. The explanation which Leriche has suggested is that there may be a reflex initiated in a peripheral cicatricial neuroma which gives rise to a chronic dilatation of the vessels just mentioned. The reflex path would then be up the peripheral nerve to the vasomotor center and out along the sinu-vertebral nerve to these vessels.

On this basis peripheral causalgia would be due to peripheral vasomotor disturbances, and those which have spread up centrally may be caused by vasomotor disturbances in the field of the radicular meningeal innervation. This appears to be at least as satisfactory an explanation as the current one of an ascending neuritis (the existence of which cannot be discovered with the microscope), or neurasthenia (which might just as well be called ascending also).

ARTERIAL LIGATION

Leriche has made a careful study of the effect of arterial ligation and resection. Not all of the findings are new, but his explanations are different. He agrees to the rise in general blood pressure after ligature of an artery. The arterial ligature is followed immediately by capillary and probably arterial contraction below the ligation, but this is soon succeeded by dilatation. An experiment demonstrating this dilatation was done as follows: In a dog the femoral artery was exposed and the corresponding knee disarticulated. The artery was then ligated without interfering with the compensatory circulation. In a short time areas of exposed muscle below the ligation began bleeding from numerous points where before the ligation neither blood nor vessel could be seen. The effect on the peripheral circulation is similar in nature to that produced by interrupting the periarterial sympathetic by adventitial stripping, although, of course, one of the blood channels is also removed.

A good while after ligation of the femoral artery in dogs, he made

arterial injections of a radiographic liquid for röntgenography. The result showed rather strikingly that the restoration of circulation is by means of anastomoses through the surrounding muscle. He believes, therefore, that capillary *dilatation* providing anastomosing channels is more important than the more orthodox anastomosis of pre-existent arteries. It is because of the difference in surrounding muscle and soft tissue that ligation of the femoral is less dangerous than ligation of the popliteal and that ligation of the subclavian is less dangerous than that of the axillary.

That arterial ligation produces a nervous effect comparable to that of sympathectomy is borne out by Halsted's observation that ligature of a subclavian aneurysm was followed by a rapid elevation of temperature in the corresponding hand six hours after the operation.[3]

Ligature of a large vessel is followed by complete restoration to normal only in exceptional cases. The troubles which are functional or trophic occasionally resemble those of Raynaud's Disease. Subsequent symptoms may be due to lack of blood supply or vasomotor disturbance. Vasomotor disturbance (nearly always after a "septic ligature") is characterized by cyanosis, stiffness, weakness and glossy skin. In such cases Leriche has found at operation that the artery was obliterated. He believes this obliteration to be due to an inflammatory process. In cases in which there was no infection he found the artery on both sides of the ligature still patent but with a lumen reduced to a size proportional to the demands made upon the stream. The sympathetic plexus in an obliterated arterial cord, being submitted to abnormal conditions, seems to cause the peripheral sympathetic spasms which are clinically seen as vasomotor disturbances. Removal of this obliterated artery without touching the functioning vessels has brought about complete cures of the vasomotor trouble in the small number of cases tried.

As for the primary ligation, he recommends that care be taken not to injure the surrounding muscle, that the peripheral artery be clamped off to prevent the passage of thrombi, that a complete exposure be made, and vessel suture done if possible, that the companion vein be ligated if the extremity appears ischemic,[4] and that the general blood pressure be raised by transfusions and other procedures.

SPINAL ANAESTHESIA

In discussing the dangers Leriche pointed out the important rôle played by the sympathetic. This form of anaesthesia in appropriate cases is preferred by him to all other methods, but, unlike Jonnesco, he does not believe in using it for all types of cases. He protests, however, against restricting its employment to those cases in which surgeons fear to use ether. He offers a well reasoned, and in many respects new, rationale for the use of

[3] Leriche showed me a large roll of manuscript which Halsted had sent him after his return from his first American visit. It contained the complete report of Halsted's unpublished work on aneurysms. It is interesting that Leriche published a study of the surgical contributions of Halsted in the *Lyon chirurgical* before the war.

[4] This was shown by Makins, Tuffier, and others during the war to be a safe and helpful procedure.

spinal anaesthesia (which he has detailed in *Lyon chirurgical*, xxi, 1924, 380–388).

Technique. The patient is wheeled into the operating room and then sits on the edge of the table for the lumbar puncture. A needle of very small bore and short bevel is used. The small bore is to prevent leakage from the spinal canal after withdrawal of the needle, and the short bevel to prevent extradural escape during the injection. Unnecessary loss of fluid is carefully prevented. The trocar of the needle is removed and a few cubic centimeters of fluid are withdrawn into a syringe. The novocaine is dissolved in spinal fluid and then injected, but this is done very slowly so as to layer the solution on the spinal fluid, "comme du vin rouge sur de l'eau"! The needle is withdrawn and the patient carried by two orderlies to the operating table. The table is then tipped so that the head is lower than the feet.

The ideal result aimed at by him is the production of analgesia without paralysis. That is, by layering the solution carefully on the fluid and immediately placing the patient on his back, Dr. Leriche states that the novocaine solution, which is slightly heavier than the fluid, reaches only the posterior nerve roots. When entirely successful, the patient has control of his limbs in the presence of complete analgesia. This is desirable for two reasons: first, the concentration of analgesic solution about the posterior roots is rendered greater; and second, the sympathetic vasomotor nerves, which pass out in the anterior roots of the thoracic cord, are spared.

Diffusion may be lessened by injecting the analgesic substance in a liquid isotonic with the cerebro-spinal fluid. In such a liquid the diffusion extends a distance of five or six roots. Hypotonic solutions should never be used. Diffusion is less when the tension of cerebrospinal fluid is greater and when the rate of injection is less.

Contra-indications to spinal anaesthesia are, for Leriche: shock, low blood pressure, inanition, and evidence of approaching syncope.

The sudden syncope and bulbar paralysis which have been so widely reported Leriche explains as due to *anemia of the bulb* rather than to penetration of the paralyzing solution to the bulb. The bulbar anemia is produced by paralysis of the vasomotor fibers in the anterior roots of the thoracic nerves, thus causing relaxation of tone in the peripheral blood vessels, particularly those of the splanchnic system. Thus, when the head is put higher than the abdomen, as is done in so many clinics, blood collects in the dependent toneless vascular bed. The tendency to bulbar anemia would explain the danger of using this method in cases of shock and perhaps in cases of hypotension. The vomiting might, of course, likewise be due to bulbar anemia.

The headaches he attributes to low pressure of the cerebrospinal fluid resulting from slow leakage of fluid into the tissues through the dural puncture hole. He successfully combats these headaches by injecting serum beneath the skin or distilled water (about 25 to 50 cc.) into a vein, to raise the cerebrospinal fluid pressure. When spinal anaesthesia is used to block the higher dorsal nerves, a pneumonitis frequently results. Leriche considers

this as probably a pulmonary vasomotor phenomenon.

Indications for the use of spinal anaesthesia are,—abdominal operations below the umbilicus and operations on the genitalia and lower extremities. For the head, face, neck and thorax the anaesthesia of choice is local analgesia and nerve-blocking. The upper abdomen in favorable cases may be attacked under spinal anaesthesia, but for the most part ether is the anaesthetic of choice.

Following spinal anaesthesia, in Leriche's experience, there is practically no abdominal distension nor vomiting, and when used on the lower spine no pulmonary complications.

In short, employ a needle of small gauge and short bevel. Lose no fluid. Inject an isotonic solution very slowly. Place the patient on his back with head lower than feet to prevent syncope. With these simple precautions, Leriche considers spinal novocaine the most innocent of all methods of anaesthesia.

To summarize briefly, Leriche has pointed out that in certain pathological conditions of the extremities the removal of the adventitial coat of the main artery of supply is followed by relief of symptoms. For justification of the operation he relies on the results rather than on a complete theoretical explanation. The cases in which relief is experienced are roughly of two main types. The first group have a circulatory disturbance due to reflex arterial spasm. (It must not be expected that the operation will restore previously obliterated vascular channels.) The second condition is causalgia or painful stump. It might be possible to speak of a third group which would include the so-called trophic ulcers and certain ill defined skin diseases.

Thanks to the work of Leriche, the nature of the changes after arterial ligation and periarterial sympathectomy are made clear. In spinal anaesthesia he has pointed out the probable rôle of paralysis of the vascular sympathetic fibers proceeding from the thoracic segments of the cord in causing the dangerous reactions to that form of anaesthesia.

A more general acceptance of the discriminating attitude of Leriche toward surgery of the sympathetic nervous system would lead to fewer unnecessary operations. He is a careful surgeon, and a good observer. He is dominated in his investigation by a well considered hypothesis. That much may be said now. Final judgment of the value of his work must be left to the future.

Post-Anaesthetic Hypoglycaemia

A Study of the Etiology of Recurrent Vomiting[1]

By Hugh Josephs

From the Department of Pediatrics, Johns Hopkins University and the Harriet Lane Home, Johns Hopkins Hospital

A SHORT time ago S. G. Ross and I reported the study of a case of recurrent vomiting (1), the salient clinical features of which were as follows: The child had had, up to the time he was studied, five attacks all of a similar nature, during which he vomited, became very drowsy and had one or more convulsions. In the last of these attacks he came under observation within two hours after the onset of his vomiting and very shortly after a convulsion. At that time his urine contained large amounts of acetone bodies, and his blood sugar was found to be 0.037 per cent. We studied the etiology of the attacks by instituting short fasting periods and by the substitution of fat for carbohydrate, and succeeded in producing hypoglycaemia and an early output of acetone bodies, without causing any symptoms other than marked drowsiness. It was evident from our findings that some factor other than withdrawal of carbohydrate was necessary for the production of an attack of recurrent vomiting, at least in the patient studied. We concluded that this other factor was probably in some way connected with the occurrence of an infection which was present in all the spontaneous attacks in our patient.

The consideration of an additional factor in the etiology of the attacks of recurrent vomiting in our patient led to the present study of anesthesia, since it is a well known fact that attacks of recurrent vomiting may occur after even very short periods of ether narcosis. Even among children who are not ordinarily subject to them, anaesthesia may occasionally precipitate attacks that are indistinguishable from those of recurrent vomiting. Some preliminary observations brought out the fact that a moderate degree of hypoglycaemia actually did occur after anaesthesia, and that this hypoglycaemia was probably more than would have occurred from an equal period of starvation without anaesthesia. In this paper it is my purpose to present evidence sufficient to establish this fact beyond any doubt and to determine the mechanism of the hypoglycaemia.

The children in this study were chosen from among those who had been submitted to a tonsillectomy. The amount of ether used in each case

[1] *Received for publication, April 15, 1925.*

was small—from one to four ounces—depending primarily on the age of the child; and the operation in all cases was of about the same duration and severity. The children were given a light supper of toast, milk, and an egg at 6 p.m. the evening before the operation, and thereafter nothing but water until after the last specimen of blood or urine had been obtained, about twenty-four hours after the operation. This entailed no hardship on the patients as they seldom wanted breakfast on the morning after operation.

The urine was obtained as far as possible in separate specimens, each representing five or six hours' excretion. This was an ideal seldom attained, as the tables will show. Acetone bodies in the urine were determined by the method of Van Slyke and reported as milligrams of β-hydroxybutyric acid. Blood was obtained by pricking the finger and the blood sugar estimated by the Fitz modification of the Bang method. Air was obtained by means of a mask fitted with Tissot valves and merely applied against the child's face. No attempt was made to obtain more than the respiratory quotient. The air was collected in a rubber bag, transferred immediately to a sampling tube over mercury and analyzed in a Haldane gas analysis apparatus.

It has been necessary to include controls in this work because starvation alone will cause a lowering of the blood sugar. These controls are the same ones used in the former paper on recurrent vomiting, and consist of five children who were fasted as a therapeutic measure in epilepsy (Table V).

In order to economize space, I have included observations in only four of the twelve cases studied (Tables I to IV). The observations that have been omitted corroborate those in the tables in essential points. Probably the most striking fact is the regular occurrence of hypoglycaemia eighteen to twenty-four hours after the anaesthesia (Tables I, II, III and IV). Hyperglycaemia is not so regular nor is there a definite connection between hyperglycaemia and the later development of hypoglycaemia (Tables II and III). Within the limits of this work there was found to be no connection be-

TABLE I

W. W. 12 years. W.

URINE						BLOOD SUGAR	R.Q.	DATE	TIME	REMARKS
Date	Time of collection	Time after anaesthesia	Duration of specimen	Creatinine per hour	Acetone bodies per hour					
		hours	*hours*	*mgm.*	*mgm.*	*per cent*				
						0.110	—	10/6/24	9.30 p.m.	
						0.106	0.78	10/7/24	8.30 a.m.	Operation 10 to 10.25; ether 4 ounces
10/7/24	8.30 p.m.	?	19	25	26	0.157	0.92		12 m.	
	11.45 p.m.	13½	3½	22	25	0.124	—		1 p.m.	
10/8/24	3.30 a.m.	17	3¾	22	27	0.103	0.79		5.30 p.m.	
						0.079	0.74	10/8/24	10 a.m.	

TABLE II

A. J. 8 years. W.

URINE						BLOOD SUGAR	R.Q.	DATE	TIME	REMARKS
Date	Time of collection	Time after anæsthesia	Duration of specimen	Creatinine per hour	Acetone bodies per hour					
		hours	hours	mgm.	mgm.	per cent				
10/11/24	8.30 a.m.	—	12	19	0	0.110	0.77		8.30 a.m.	Operation 9 to 9.20 a.m., 1½ ounces ether
						0.111	0.82		11.30 a.m.	
	4 p.m.	7	7½	17	32					
	8.30 p.m.	11½	4½	19	52	0.106	0.81		5 p.m.	
10/12/24	2.30 a.m.	17½	6	20	37					
	8 a.m.	23	5½	20	275	0.073	0.72	10/12/24	11 a.m.	

TABLE III

L. J. Age 5 years. Col.

URINE						BLOOD SUGAR	R.Q.	DATE	TIME	REMARKS
Date	Time of collection	Time after anæsthesia	Duration of specimen	Creatinine per hour	Acetone bodies per hour					
		hours	hours	mgm.	mgm.	per cent				
10/14/24	8.30 a.m.	—	6½	14	0	0.101	0.85	10/14/24	8.30 a.m.	Operation 2.10 to 2.20 p.m.; ether 2 ounces
	6 p.m.	4	10	13	Tr.	0.101	0.73		3.30 p.m.	
10/15/24	3 a.m.	13	9	14	27	0.079	0.69		8 p.m.	
	11 a.m.	21	8	14	44	0.065	0.70	10/15/24	10.30 a.m.	

TABLE IV

C. Z. 8 years. W.

URINE						BLOOD SUGAR	R.Q.	DATE	TIME	REMARKS
Date	Time of collection	Time after anæsthesia	Duration of specimen	Creatinine per hour	Acetone bodies per hour					
		hours	hours	mgm.	mgm.	per cent				
3/21/25	2.15 a.m.	—	4½	19	0					
	9.45 a.m.	—	7½	20	Tr.	0.112	0.72	3/21/25	8.15 a.m.	Operation 11.15 to 11.45, ether, 4 ounces
	3.30 p.m.	4	5¾	19	50	0.160	0.81		2.15 p.m.	
	7.30 p.m.	8	2(?)	17(?)	24	0.117	0.84		7.30 p.m.	
3/22/25	6 a.m.	18	12(?)	19(?)	29					
	10.30 a.m.	23	4½	20	416	0.073	—	3/22/25	9.30 a.m.	

tween the duration of anaesthesia, the amount of ether used, or the severity of the immediate reaction, and the later development of hypoglycaemia. That is, the degree of hypoglycaemia seems to depend on something inherent in the metabolism that is called into being by the anaesthetic and which is, at least after short periods of anaesthesia, a function of the child and not of the anaesthetic.

The excretion of acetone bodies which in the majority of cases is definitely greater than that which occurs after a similar period of simple starvation (Tables II and IV) is of especial interest because of recent reports that acetonaemia does not occur after ether anaesthesia to any considerable extent (2). Those who have looked for it have either examined the blood a few hours after the anaesthesia or have examined an entire twenty-four hour specimen of urine. I think the results here will show clearly enough why negative results have been obtained by these methods. In Table I, for instance, the failure to obtain an increase in acetone body excretion is due to the fact that the last urine specimen was obtained too early. That increase in acetone body excretion is not invariable, however, is shown by the data in Table III.

The uniformity of these results would seem to be sufficient to establish the fact that the metabolic processes following anaesthesia are such as to cause hypoglycaemia, that this hypoglycaemia is accompanied by an increasing excretion of acetone bodies and that the hypoglycaemia and acetone body excretion is more than one would expect from simple fasting.

An attempt to explain the mechanism of hypoglycaemia led to the determination of the respiratory quotient. It must be remembered, however, that results obtained by a method as crude as the one that I have used are valuable only when the variations on which conclusions are to be based are sufficiently great and always occur in a single direction. The figures in the tables are sufficient on this point, I think, for there is a parallelism between the rise in blood sugar and the rise in respiratory quotient that is quite striking, so much so that it suggests some

TABLE V

Fasting controls

NAME	AGE	BLOOD SUGAR		ACETONE BODY EXCRETION		
		4 hours	40 hours	First twenty-four hours	Second twenty-four hours	Third twenty-four hours
D. T.	4	0.110	0.085	0	Tr.	224
M. C.	12	0.125	0.100	—	—	210
M. W.	12	0.105	0.083	—	—	—
R. V.	9	0.101	0.072	0	Tr.	48
J. McD.	5	0.117	0.098	0	100	278

causal relation between the two. My data at present are insufficient for the discussion of this interesting point; all I can say now is that, if any such relation exists, it is not quite so simple as might appear from the figures in the tables. A more important consideration for this paper is to show that a rise in the respiratory quotient as I have obtained it is a true measure of a rise in utilization of carbohydrate.

Changes in the CO_2 content of the serum, which occur during and immediately following anaesthesia, are such as to render an uncorrected respiratory quotient valueless as a measure

of the oxidation of carbohydrate. It would be out of place to do more than sketch the mechanism of acidosis during anaesthesia as determined by a number of workers (3). These changes are dependent primarily on two occurrences: (*a*) the pressure of CO_2 in the blood stream rises, probably because of a decreased sensitiveness of the respiratory center; (*b*) some acid, possibly lactic, is formed during anaesthesia, that causes a diminution in the bicarbonate content of the plasma. Thus the ratio of free to bound CO_2, or of undissociated H_2CO_3 to HCO_3-ion, upon which the hydrogen-ion concentration of the plasma depends, is increased and the plasma consequently becomes more acid. When the anaesthesia is stopped, both the above processes are reversed: (*a*) the CO_2 pressure falls quickly as the respiratory center regains its sensitiveness and causes an increased pulmonary ventilation to remove the excess of CO_2 from the plasma; (*b*) the disappearance of the abnormal acid by excretion, oxidation or other means causes a gradual rise in the bicarbonate of the plasma. After short periods of anaesthesia the first process is completed in about half an hour, when the ratio of the free to bound CO_2 has reached a normal value and the hydrogen-ion concentration of the plasma has therefore also become normal. The second recovery process is slower, so that at the end of half an hour the concentration of bicarbonate is still below its normal value, but is increasing slowly. Inasmuch as the ratio of free to bound CO_2 must remain constant with a constant hydrogen-ion concentration, as the bicarbonate, or bound CO_2, rises there must be a rise also in the free CO_2 and therefore in the total CO_2 contained in the plasma. This is accomplished by a retention in the plasma of CO_2 formed in metabolism. It is thus seen that immediately following the cessation of anaesthesia respiratory quotients will be too high, while after half an hour, or an hour at most, they are apt to be too low.

The earliest respiratory quotients following anaesthesia in this work were obtained two hours after its cessation, so that it is altogether likely that these values are, if anything, too low. For this reason I have no hesitation in using a rise in the respiratory quotient following anaesthesia as a true indication of a relative increase in the use of carbohydrate. An objection that might be raised is that the rise in respiratory quotient is not due to an increase in use of carbohydrate but to a decrease in the use of fat. A determination of this point would require collection of the total expired air, a procedure I have found to be impracticable in this work with children. A determination of the total respiratory exchange in a fasting dog showed that the resting metabolism as well as the respiratory quotient actually rose after anaesthesia. Without going into the details of this determination, I can say that this rise was not due to increased activity or chilling of the animal or to rise in temperature of the animal.

A further finding confirming the idea of a rise in carbohydrate oxidation is the behavior of the acetone bodies in C. Z. (Table IV). Acetone bodies due to fasting had already begun to appear before the anaesthetic was given. Following the anaesthetic

these acetone bodies practically disappeared from the urine until 8 to 10 hours later after which they increased rapidly. The period of disappearance of acetone bodies from the urine may be seen to correspond roughly to the period of increased respiratory quotient, *i.e.*, of greater utilization of carbohydrate.

The considerations just outlined have led me to believe that an increase in the respiratory quotient is at least a rough measure of an increase in the use of carbohydrate. From the work so far I may conclude that anaesthesia may cause an increased oxidation of carbohydrate lasting for several hours, that this increased oxidation is usually accompanied by hyperglycaemia, the degree of which more or less parallels the extent of the increased oxidation, and that it is likely that the increased oxidation of carbohydrate is at least partially responsible for the hypoglycaemia that occurs twelve to eighteen hours later.

The bearing of this work on the etiology of the attacks of recurrent vomiting that follow anaesthesia hardly needs a more detailed discussion than has been given at the outset of the paper. I was fortunate enough to be able to study a child (A. V.) who on the day following the anaesthesia had an attack of vomiting, became quite drowsy, and clinically appeared to be suffering from the so-called "post-anaesthetic acidosis," a condition which, as has been said, is often indistinguishable in its clinical characteristics from an attack of recurrent vomiting. The results in this case are contained in Table VI. In the first place, the blood sugar began to drop after only twelve hours fasting, a result none of the others showed; in the second place, the blood sugar and respiratory quotient remained above the normal for a longer time than in any of the other children; and in the third place, the blood sugar reached a lower level and the excretion of acetone bodies a far higher level than in any of the other obser-

TABLE VI

A. U. 8 years. W.

URINE						BLOOD SUGAR	R.Q.	DATE	TIME	REMARKS
Date	Time of collection	Time after anaesthesia	Duration of specimen	Creatinine per hour	Acetone bodies per hour					
		hours	hours	mgm.	mgm.	per cent				
						0.110	0.81	11/17/24	9.30 p.m.	
11/18/24	9.30 a.m.	—	4½	19	0	0.088	0.74	11/18/24	9 a.m.	
	11 a.m.	—	1½	17	Tr.					Operation 3 to 3.20 p.m.
	7.45 p.m.	4½	8¼	21	75	0.098	0.76		5 p.m.	
11/19/24	1.45 a.m.	10½	6	20	135	0.128	0.88		8 p.m.	Ether, 3½ ounces
	6.45 a.m.	15½	5	20	248					
	12.15 p.m.	21	5½	21	780	0.070	0.70	11/19/24	12 m.	
	5.15 p.m.	26	5	18	1087	0.061	—		4 p.m.	

vations. The results in this case suggest the hypothesis that increased use of carbohydrate following anaesthesia bears an important part in the etiology of an attack of post-anaesthetic acidosis and by inference of an attack of recurrent vomiting occurring after anaesthesia.

Do we have to look further for the etiology of recurrent vomiting than in an uneconomical use of carbohydrate? Did the child burn her carbohydrate prodigally to the point of exhaustion merely because it was there to be burned, while other children were more sparing in their use of it and so escaped the dangers of carbohydrate exhaustion? Or do we have to look further for some more fundamental cause for the increased oxidation of carbohydrate? These are questions that must be left for the future, when we shall have more knowledge of the factors which control the oxidation of carbohydrate.

SUMMARY

The curve of blood sugar, the excretion of acetone bodies, and the respiratory quotients have been studied in a series of children who had undergone short periods of anaesthesia. Hypoglycaemia of moderate degree was found to occur with great regularity eighteen to twenty-four hours after the anaesthesia. This hypoglycaemia was preceded usually, but not invariably, by an increase in the respiratory quotient and was accompanied by an increased excretion of acetone bodies in the urine. These changes were greater than could be accounted for by starvation. The bearing of these results on the etiology of recurrent vomiting and the allied condition of "post-anaesthetic acidosis" is discussed.

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Experimental Study of Amyloid Formation

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TECHNIQUE

With Kuczynski's discovery of the method of producing amyloid artificially in white mice by injecting nutrose (sodium caseinate) or by feeding the animals with cheese, the possibility of investigating this mysterious substance passed into a new stage. It is now possible to produce every degree of amyloidosis experimentally, whereas previously the result of the experiment was more or less uncertain and could not be predicted.

The technique is simple: 0.4 to 0.6 cc. of a 5 per cent sterile solution of nutrose (Meister Lucius u. Bruening, Hoechst am Main) is daily injected intramuscularly in the gluteal region. It is not necessary to shave the hair in this region, but it seems advisable to change the sides daily and to clean the skin before the injection with 80 per cent alcohol. A similar result is obtained by continued feeding with cheese. The Swiss cheese, which the mice preferred to any other kind, proved to be the best material. In addition to the cheese a small portion of bread was given daily in order to prevent their aversion to cheese alone. By feeding in this manner it is easy to produce amyloid.

The time for the development differs. After about thirty injections of nutrose almost every mouse develops amyloid. The very first result was obtained after 17 injections and the first result in the feeding series after 30 days. Older animals seem to react more easily and more quickly and therefore it is more profitable to take litters of animals in order to regulate to a certain extent the duration of the experiment.

The beginning of the change is recognisable by the appearance and the behavior of the mice; the hair becomes shaggy and they are strangely passive and listless—quite a contrast to their normal state. After continued injections the mice become very ill and die shortly afterwards, showing extreme degrees of amyloid in all the parenchymatous organs. It is not difficult to identify the amyloid in the advanced stage; the iodine method shows it sufficiently and the aniline dyes also react with metachromatism, so that there can be no doubt about the nature of the pathological changes in the organs. Also, without specific stains the amyloid in this stage is easily recognised by its peculiar structure which is crystalline in appearance; this fact was discovered and described by Kuczynski.

The best staining of old amyloid is obtained with the method described by Bennhold (stain with a 1 per cent

solution of Congo-red and heat, wash, put slide in saturated lithium-carbonate for about 20 seconds, and differentiate with 80 per cent alcohol). The advantage of this method is that one may stain amyloid in paraffin sections which can be cleared, in this way making it possible to study its relations to the surrounding tissue.

It is true that the thicker fibrils of elastic tissue, especially of that around the arteries, are also stained; but the shade of the elastic tissue is more brownish-red, while that of amyloid is pinkish-red; hence, it is unnecessary to exaggerate the differentiation which may cause the decolorization of less deeply stained amyloid.

The nuclei can be stained with haematoxylin; after a short differentiation in acid alcohol the slide is washed in water and put in a saturated solution of lithium-carbonate, whereby the pinkish-red color of the amyloid is very nicely contrasted with the deep blue of the nuclei.

The proof or demonstration of young and youngest amyloid is more difficult. The iodine method shows very poorly, if at all, and the metachromatic reaction cannot be obtained in formol-fixed material nor even in unfixed frozen sections. The Congo-red method fails likewise. In this stage the crystalline structure is also very difficult to find.

In order to obtain a positive reaction at all, it is necessary to fix the material in alcohol and to stain paraffin sections with methyl-violet for metachromatism. But this method is also unsatisfactory: the sections cannot be cleared, which makes the distinction between amyloid and the organ tissue almost impossible.

In order to improve these methods, we tried to inject Congo-red into the blood-stream of the mice, thus repeating the experiment of Bennhold, who discovered in this way in human beings the specific staining capacity of Congo-red for amyloid. The results obtained by this method prove that this vital staining is the ideal method for demonstrating amyloid, especially young amyloid. If one succeeds in getting 0.5 cc. of a 1 per cent solution of Congo-red into the blood-stream, and if the animal lives from one to two hours after the injection, all the amyloid, even the youngest, is stained red, while the rest of the tissue remains unstained.

The successful injection causes an immediate pinkish-red coloring of the mucosa of the nose and mouth. About two hours after a successful injection into a normal animal, the color has already been excreted. In formol-frozen sections, which are made immediately after the autopsy, only the trabeculae of the spleen show a slight pinkish coloring. In doubtful cases this fact can be used as proof of the success of the injection.

Moreover, traces of the injected fluid can be found in the lumen of the intestines, and in some of the capillaries of the kidneys, as a brownish-red content. Also, the urine may show a pinkish color.

In frozen sections of injected mice suffering from amyloidosis, every trace of amyloid is stained bright red. After staining the nuclei with haematoxylin, the slide may be cleared and mounted in balsam.

Formol fixation is not indicated for paraffin sections. The color of the Congo-red vanishes in formalin after a few hours. If it is absolutely neces-

sary to use formalin-fixed material, parts of the organs are heated for a short time in 10 per cent formalin solution; immediately afterwards the formalin must be washed out in running water for about 24 to 48 hours. After rapid embedding, the color still shows sufficiently. Much better results are obtained from Zenker fixation without formalin; the color of the Congo-red changes to a more brownish-red, but the vital stain is very well preserved.

By far the best results are obtained after fixation in cold saturated sublimate solution, as far as the shade and the intensity of the Congo-red color is concerned. In cases of young amyloid it seems advisable to use this kind of fixation exclusively. The tissue is fixed in this solution for about 24 hours, dehydrated in ascending alcohol, and then embedded. After dissolving the paraffin of the sections, the sublimate precipitations are dissolved by iodine tincture or Lugol's solution. The iodine stain is washed out in alcohol or sodium thiosulphate. The nuclei are slightly tinted with haematoxylin; after the color of the haematoxylin has turned deep blue, the section is dehydrated in ascending alcohol, cleared in xylol and mounted in balsam.

Sections with vitally stained amyloid can be used for Weigert's elastic-tissue stain; in this case it is better not to stain the nuclei at all.

In order to demonstrate fat and amyloid in the same section, we tried to stain the fat with chlorophyl, after the method described by Eisenberg. No positive result was obtained with chlorophyl (Merck), and chlorophyl from G. Hell & Company, used and recommended by Eisenberg, was not available.

Indophenol stains fat very unsatisfactorily, so that we finally used Sudan III, a color which differs enough from the Congo-red.

For the impregnation of the connective-tissue fibrils after the method of Maresch-Bielschowsky, the tissue is heated for a short time in 10 per cent formalin; afterwards the formalin is washed out in running water for 24 hours and then in distilled water for 24 hours. The tissue is frozen and impregnated in the usual way, but the sections should not be left in the reducing 20 per cent formalin solution too long. In slightly stained sections the pinkish color of the amyloid is still recognizable. Moreover, rapidly embedded formol-paraffin sections occasionally still show the color of the vitally stained amyloid after the silver impregnation. But, just after silver impregnation the crystalline structure is so easily seen that it seems almost unnecessary to color the amyloid.

The Maresch-Bielschowsky method seems to be the only one suitable for the isolated demonstration of amyloid and fibrils. Van Gieson stains only the thicker connective-tissue fibrils; the Mallory and Verocay stains show the amyloid and the connective tissue in the same color, so that the finer differentiation is impossible.

RESULTS

The main object of the following study was to demonstrate the very early stages in the development of amyloid and the changes in the organs before and during its appearance.

In order to obtain a regular series, animals of the same age were injected,

or fed daily with the same amount of nutrose and killed at regular intervals, animals which died spontaneously being discarded, while one untreated mouse was kept as a control in each series.

Vital staining of the amyloid was carried out in most cases as described above. The organs of the animals, when killed, were fixed immediately in sublimate, Zenker and absolute alcohol, and a part boiled in 10 per cent formalin for frozen sections.

Since the results of injections of nutrose were found to be more constant and readily predicted than those of feeding, the former method was preferred.

After about ten or fifteen injections the liver and spleen are found to be enlarged, the spleen to three or four times its normal size, so that the change is recognized at once on opening the abdomen. Its capsule is stretched and its consistence increased.

The normal liver of the mouse shows in a periportal field only a little connective tissue around the vein, so that the liver cells lie close to its endothelial lining and the capillaries are narrow and irregularly lined with endothelium. After from 1 to 15 injections there are found dense accumulalations of cells which Kuczynski regarded as immature blood cells in the periportal spaces about the portal veins or bile-ducts, or extending out into the capillaries of the liver lobule. There are large and small mononuclear cells and many giant cells with one or more nuclei. Polymorphonuclear leucocytes are rarely seen. The liver cells are severely damaged. The nuclei are vacuolated and in the cytoplasm dense masses alternate with vacuoles either at the periphery or just about the nucleus. The vacuoles do not contain fat, although the amount of fat in the liver cells may be increased.

In the normal spleen the follicles are widely separated by the paler pulp, in which there are relatively few cells, although occasional giant cells may be seen. After the 10 or 15 injections the Malpighian bodies are greatly enlarged and coalesce into anastomosing strands which do not stand out sharply from the intervening pulp because of the great increase in the number of the cells in the sinuses of the pulp. There are accumulations of such cells scattered through this tissue, and among them the great number of giant cells is especially striking.

The kidneys and intestines show no marked changes at this time.

Intravenous injections of Congo-red fail at this stage to give any trace of amyloid, and sections from alcohol-fixed tissue show no metachromatic reaction.

The first sign of red-staining with Congo-red was seen in the liver of a mouse which had received 20 injections of nutrose. The liver cells showed the vacuolar degeneration described above, and the cellular infiltration in the periportal spaces and in the capillaries of the lobules was well developed. In these periportal fields, sometimes in direct contact with the wall of the vein, there were pink-stained patches, always with a small homogeneous pale pink central area with pyknotic nuclei about its margin or even occasionally in its interior. Some of these nuclei stain very badly and give the impression of being gradually dissolved. A crystalline structure

was seldom suggested in the stained areas. The position of such areas corresponds with that of the more advanced stages of amyloid, and in mice injected for two days longer typical amyloid was found in the liver, spleen, intestines and kidneys.

In its definite early stages amyloid appears in the liver in the periportal tissues where the small particles produce no reaction and show a typical crystalline structure staining bright red after an intravenous injection of Congo-red. Damaged nuclei are found about them. The tissue about the portal veins is widened, with swollen and vacuolated cells and thick, shining, undulating fibrils.

After a few more injections, almost every periportal field shows amyloid partly or completely surrounding the vein, and at the same time the amyloid appears also in the intermediate zone of the lobule. The perivascular amyloid has a peculiar structure showing undulating, ridge-like lines with very fine fibrillae perpendicular to these lines. In the capillaries it has a more star-like crystalline structure and is separated from the liver cells only by flattened cells with fusiform nuclei; these show all stages of degeneration and shrinkage with pyknotic fragmented nuclei until they finally disappear.

About the vessels the amyloid lies next the vessel wall or sometimes scattered in the area of infiltration of cells. So, too, in the capillaries it may be in the center of an accumulation of cells, next it in the same capillary or above in a neighboring capillary (Fig. 3). The liver cells may appear narrow and atrophic even when cell accumulations alone occupy and widen the capillaries.

The amyloid certainly does not cause these infiltrations because they are found in similar places long before the appearance of amyloid and because the oldest amyloid about the vessels does not produce new infiltrations but lies without reaction in the tissue.

In two of the animals a peculiar condition was found in the liver. Amyloid had formed about the portal venules, sometimes completely surrounding them—in other places incompletely; numerous giant cells were also found in the immediate neighborhood of the portal veins, but only in those places where there was no amyloid or where the amyloid ring was incomplete. Such giant cells grow larger, their protoplasm becomes homogeneous and their nuclei fade away, being then recognizable only as shadows near the periphery as though they were gradually dissolved in the protoplasm. In the center, sometimes in the periphery, there appear irregular shining lines of flecks which gradually increase in size and finally occupy the whole space within the dying giant cell (Figs. 1 and 2). One of the animals was injected with Congo-red intravitally, the other received no injection. Since both show precisely the same histological picture in the liver, there is no reason to suppose that the giant cells were produced by the Congo-red, nor does the appearance of amyloid in the giant cells suggest phagocytosis. Stained pink in the early stages, the red color grows deeper and a crystalline structure becomes obvious in the later stages.

It seems that in these giant cells one may see on a large scale the formation of amyloid which occurs almost unnoticed in the smaller cells.

In late stages in the development of amyloid when this substance occupies the familiar position about the portal vessels and extending in trabeculae into the lobule, silver impregnation by the method of Maresch-Bielschowsky shows that the network of fibrils about the vessels can no longer be demonstrated except in places where the amyloid is lacking. They are either displaced or stain but faintly or not at all.

When the elastic tissue is stained, it is found that the cells accumulated in the periportal field are for the most part outside the elastic wall of the vessel, although a few are included between the fibrils. But when amyloid has appeared, the elastic fibrils are interrupted by the amyloid which may appear sometimes as a continuation of them (Fig. 4). In late stages the elastic tissue is greatly reduced or lacking about some vessels, or there may still be very fine fibrils outside the amyloid ring.

In the spleen the amyloid appears at about the same time as in the liver, first in the periphery of a follicle in the form of fine anastomosing trabeculae, or in masses with crystalline structure. Usually, the formation of amyloid progresses more rapidly in the spleen than in the liver, and the anastomosis of the trabeculae of amyloid about the follicles produces the typical sago spleen.

In early stages in animals stained with Congo-red there are unstained, homogeneous-looking areas around the Malpighian bodies where amyloid might be expected. In these areas traces of nuclei or the outline of cells may be recognizable, or there may be faintly stained nuclei surrounded by a vacuole-like space in a sort of syncytium. Such unstained areas often form the continuation of distinctly red-stained amyloid which radiates into them in fine processes (Fig. 5). In other places, fine fibrils of amyloid appear in necrotic cell masses in intimate relation with disintegrating nuclei.

The fibrils of the reticulum are distinctly enlarged and swollen. Numerous giant cells lie between and near the amyloid masses, some of them preserved in the midst of the amyloid, while others have lost their nuclei and appear as masses of protoplasm with processes. Even this may be partly replaced by amyloid and connected with surrounding amyloid. In other cases lines of nuclei lying close to the amyloid represent the remains of a giant cell. In one of the cases mentioned above giant cells with intracellular amyloid were found also in the spleen (Fig. 6). The transition of the protoplasm to the intracellular amyloid is very gradual, so that one does not receive the impression that the amyloid has been phagocytized by these giant cells.

Silver fails to impregnate the fibrils about the follicles where there is amyloid, whereas the supporting tissue of the Malpighian bodies, the trabeculae and the fibrils of the capsule are deeply stained. No amyloid could be found apposed to the fibrils even in the earliest stages but the fibrillar network may be interrupted by the amyloid.

In the kidneys amyloid is produced by injections later than in the liver and spleen, although after feeding with cheese it is found in all these organs at the same time. In animals injected with nutrose the first trace of it is seen in the medulla near the papilla where it forms delicate red-stained lines

in the walls of the capillaries beneath the endothelial cells. The tubules are found surrounded by fine rims of amyloid which anastomose with the reticulum surrounding the neighboring tubules. This reticulum is swollen and many of the nuclei have disappeared.

In the glomeruli capillary loops appear swollen just before and during the appearance of the amyloid and their walls seem to assume the form of a syncytium in which the nuclei perish and disappear. Amyloid appears in the form of lines or irregular patches which soon develop a crystalline structure (Fig. 7).

Brightly stained amyloid is found in the medulla in patches which stand out prominently on account of their color and glassy structure. In these areas the contours of some cells are still recognizable, with fragments or shadows of nuclei. Occasionally tubules are partly or entirely included.

In the intestine the amyloid appears first in the walls of the small vessels at the bases of the villi or in the tissue between the glands. Later these fine lines anastomose, and amyloid appears at the tips of the villi. Finally, almost the whole framework of the villi consists of amyloid with crystalline structure.

DISCUSSION

Two recent papers expressing different views show that the genesis of amyloid is still a matter of controversy.

Frank (*Ziegler's Beiträge*, 1920, lxvii, 181) produced amyloid by the injection of living or dead Friedländer's bacilli and also with pneumococci and staphylococci. He concludes that the formation of amyloid is due to the direct action of bacteria. Different bacteria have the faculty of producing this effect, which is to be regarded as the result of a bacterial intoxication and not necessarily of an infection in the narrower sense.

The amyloid is to be interpreted as the result of coagulation of the protoplasmic albumen with a swelling of the protoplasm of the cells of the vessel wall, that is, the smooth muscle and especially the connective tissue cells and fibrils, the coagulation being aided by the acid formed by the bacteria. In addition, the protoplasm of red corpuscles and other cells absorbed in the vessel wall and connective tissue, in so far as it is capable of swelling, may take part in this "*Schwellungsnekrose.*" Theoretically, it is undoubtedly to be assumed that blood and lymph plasma may take part in this coagulation and swelling under the influence of the same non-specific bacterial endotoxin, but no evidence in this direction could be obtained from animal experiments.

Kuczynski (*Virchow's Arch.*, 1923, ccxxxix, 185) produced amyloid in white mice by feeding cheese or by injecting nutrose, thus excluding any possibility of infection. He disagrees with Frank as to the taking up of the plasma albumen of phagocytized blood corpuscles by the amyloid masses and states that the picture, which Frank describes as the first appearance of amyloid, doubtless represents no more than a bacterial necrosis.

He adopts the theory of infiltration and concludes that "the amyloid is a peculiar form of crystalline excretion of decomposed albuminoid substances outside the vessel wall. This process may perhaps be due to coagulative substances of the tissue or of the layers of the vessel wall active probably out-

side the intima. There is probably a direct connection between the decomposed substance and the absorbed or parenterally injected substance, since amyloid degeneration as such cannot be accepted with certainty."

Hence the conclusion seems possible that the amyloid is simply due to an accumulation of abnormally circulating albuminoid substances of various origin precipitated in crystalline form outside the vessels.

Kuczynski's method, of course, excludes the complication of bacterial action in the formation of amyloid. But although in most cases, even including those in the earliest stages, one sees histological pictures which might suggest an infiltration, our many observations of intimate relations between destructive changes in the cells and consequent and intimately related appearance of amyloid cannot be explained in this way. The swelling of the reticulum in the parenchymatous organs before and during the appearance of amyloid, the formation of giant cells in the periportal spaces of the liver with subsequent swelling of their protoplasm and disintegration of their nuclei, and the appearance of amyloid in such altered cells, the accumulation of cells in the capillaries of liver and spleen with necrosis, fragmentation of nuclei, fusion of protoplasm and gradual appearance of amyloid are all inconsistent with the idea of a simple perivascular deposition of circulating albuminoid substances. So, too, the similar appearance of amyloid in giant cells in the spleen, in the glomeruli of the kidneys after their capillary loops have become swollen and have lost their nuclei, and in the areas in the medulla of the kidney in which debris of cells and nuclei can still be demonstrated during all stages of development of amyloid, which becomes tinctorially more and more distinct. These appearances, as well as the destruction of connective tissue and elastic fibrils in areas in which amyloid is appearing, go to show that the amyloid is developed at the expense of the tissue rather than by mere infiltration. The genesis of amyloid can be studied only in these early stages, since the evidence of its mode of formation is lost in the late stages in which amyloid has lain for a long time in the tissues. In the tissues of human beings it is rare to find such early stages—the process goes on slowly and gradually and its beginnings must lie far back as compared with what one may see in an animal in which the new formation is rapidly forced.

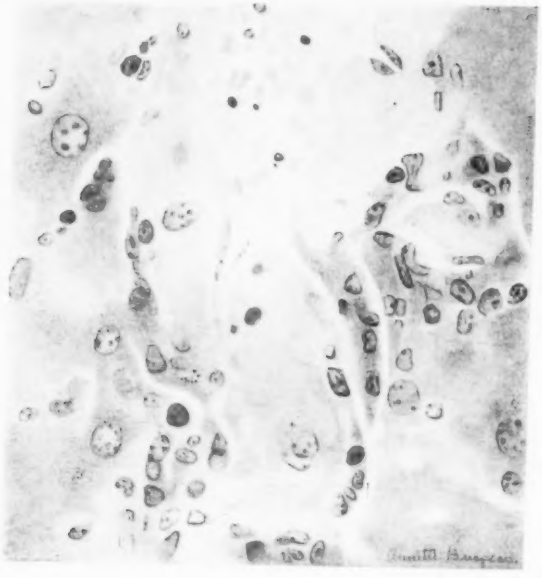
If we compare the amount of nutrose injected with the body-weight of the mouse, we find that an average mouse weighs about 20 grams; 0.6 cc. of a 5 per cent solution of nutrose weighs 0.606 grams, so that after 33 injections one has introduced an amount of the solution equal to the body-weight of the mouse. It is difficult to imagine that a human being even with the most profuse suppurative process could absorb a comparable amount of damaging substance in a similar period.

CONCLUSIONS

1. Kuczynski's method of subcutaneous injection of nutrose produces amyloid with certainty.

2. The earliest stages in the formation of amyloid are readily demonstrated only by vital staining with intravenous injections of Congo-red.

3. Severe damage to the cells of the



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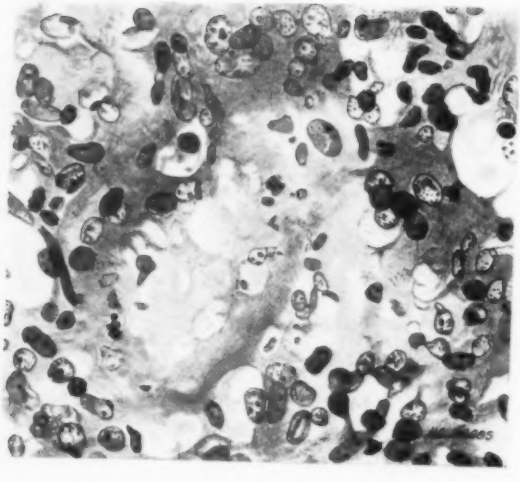

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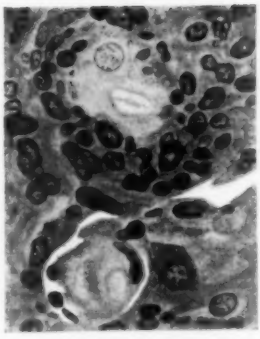

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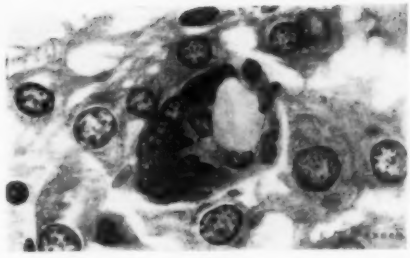

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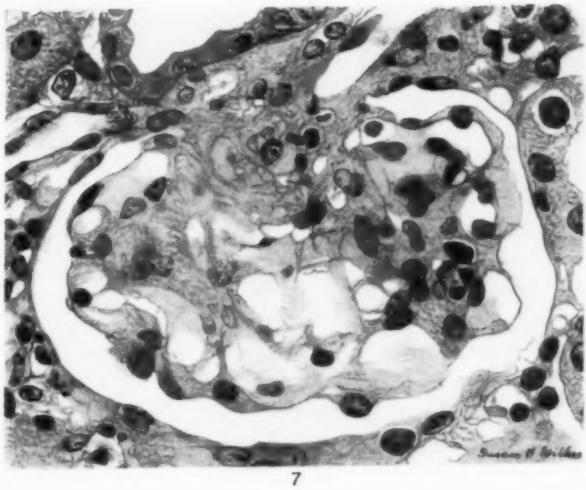

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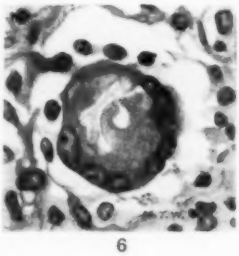

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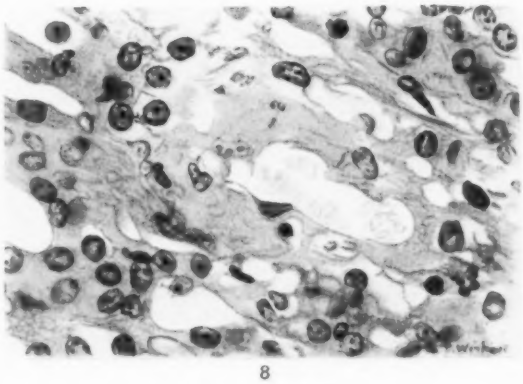

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parenchyma, infiltration of cells in the periportal regions and in the capillaries of the liver, increase in the cells of the Malpighian bodies of the spleen and cell infiltration about them are constantly seen before and during the appearance of amyloid.

4. Similarly there is destruction of connective tissue and elastic fibrils in the areas where amyloid appears.

5. Before the appearance of amyloid in parenchymatous organs there is a swelling of the cells of the vessel walls, and of the connective tissue and fibrils of the reticulum with dissolution of the nuclei and fusion into a formless mass in which the first traces of amyloid appear.

6. The process is particularly well seen when the formation of amyloid is most rapidly forced.

7. The assumption of bacterial cooperation in the production of amyloid is unnecessary.

I wish to acknowledge my appreciation of the very valuable assistance of Dr. G. Riehl.

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EXPLANATION OF PLATE 1

FIG. 1. Periportal field of amyloid liver. Giant cell with homogeneous protoplasm and vanishing nuclei. Cell syncytium. Early amyloid appearing in protoplasm of giant cell and cell syncytium.

FIG. 2. Section of liver with early amyloid. The giant cell above still shows a nucleus; there is a shining line in the homogeneous protoplasm which does not stain with Congo-red. The giant cell below no longer contains nuclei. There are two lines of young amyloid stained with Congo-red in the homogeneous protoplasm which show a crystalline structure. There is a cellular infiltration around these giant cells.

FIG. 3. Liver with early amyloid. Intracapillary amyloid, showing crystalline structure. The amyloid is surrounded by cells.

FIG. 4. Elastica stain of a periportal vein in an amyloid liver, vitally stained with Congo-red. Interruption of elastic fibres by amyloid.

FIG. 5. Amyloid spleen. Necrobiosis of splenic cells, formation of syncytium which gradually changes into amyloid.

FIG. 6. Amyloid spleen. Splenic giant cell with intracellular amyloid.

FIG. 7. Amyloid kidney. Glomerulus showing necrobiosis of cells, formation of a homogeneous matrix and appearance of early amyloid in this matrix.

FIG. 8. Amyloid kidney. Necrobiosis of cells in medulla before the appearance of amyloid. (Oil immersion.)

The Effect of Parathyroid Extract and Liver Extract on the Hypertension Produced by Guanidine Compounds

By Ralph H. Major and C. R. Buikstra

From the Department of Internal Medicine, University of Kansas School of Medicine, Kansas City.

In several previous communications (1, 2, 3), attention has been called to the marked pressor effect of guanidine bases, and observations have been made upon various substances which influence this action. This pressor effect is remarkable for its duration, the elevation in blood pressure often lasting four or five hours.

The guanidine bases have been studied especially by Noël Paton (4) and his coworkers, whose observations suggest that they may be the toxic substances responsible for idiopathic tetany and for tetany parathyreopriva. Because of this supposed relationship we thought it would be of interest to study the effect of parathyroid extract upon the hypertension produced by guanidine compounds. Such experiments were made possible through the kindness of Eli Lilly and Company, who supplied us with two batches of parathyroid extract, one prepared by the method of Hanson (5) and the other, by the method of Collip (6).

In five control experiments the effect of these parathyroid preparations upon the normal blood pressure was studied. Fig. 1 shows that parathyroid extract prepared by the method of Hanson, when injected intravenously in the amounts used in these experiments, has no effect upon the normal blood pressure of a dog. Fig. 2 shows a similar experiment carried out with the parathyroid extract of Collip. These experiments indicate that parathyroid extract in doses of from 5 to 10 cc. has no effect upon the normal blood pressure.

Ten experiments were carried out to study the effect of parathyroid extract upon the hypertension produced by guanidine compounds. Five of these were carried out with Hanson's extract and five with Collip's preparation. Methyl guanidine sulphate, in doses of 0.1 gm. per kilo of bodyweight, was employed, and injections of parathyroid extract were made on an average of five minutes after the elevation in pressure. Two typical tracings are shown in Figs. 3 and 4. In all of the experiments the injection of parathyroid extract produced a prompt fall in blood pressure.

Since both Hanson's parathyroid extract and that of Collip contain hydrochloric acid, the effect of the

FIG. 1. FALL IN BLOOD PRESSURE PRODUCED BY LIVER EXTRACT ADMINISTERED THROUGH STOMACH TUBE

Blood pressure elevation produced by intravenous injections of methyl guanidine sulphate

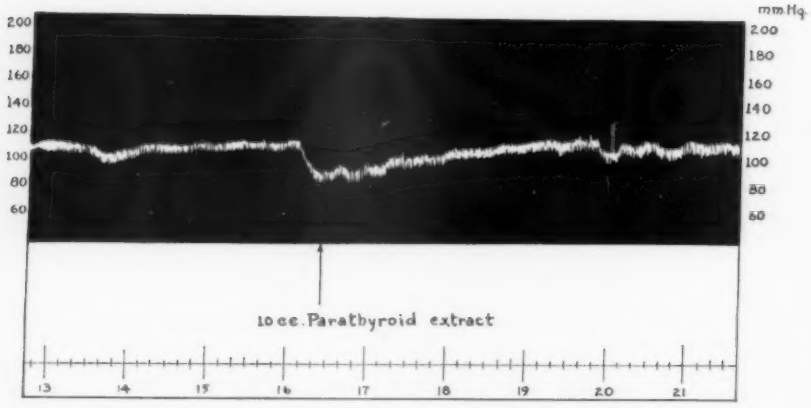

FIG. 2. EFFECT OF CHOLINE HYDROCHLORIDE UPON HYPERTENSION PRODUCED BY METHYL GUANIDINE SULFATE
Injections intravenous

injection of this amount of hydrochloric acid was studied. It was found, however, that in order to produce a lowering of the hypertension produced by guanidine it was necessary to inject more than ten times the amount of hydrochloric acid contained in the doses of parathyroid extract.

In another series of ten experiments we studied the effect of liver extracts upon the hypertension produced by methyl guanidine sulphate. This extract was prepared with hydrochloric acid by a method similar to that employed in the preparation of the parathyroid extract. These liver extracts produced a prompt fall in the blood pressure, this fall being to normal and remaining so for the duration of the experiment.

The liver extracts were found to lower somewhat the normal blood pressure of dogs, the action being quite as prompt as that of amyl nitrite and also, like that of the latter, rather temporary (Fig. 5). Unlike amyl nitrite, however, which produced only a transitory effect upon the high blood pressure caused by guanidine compounds, the liver extracts exerted an effect on this high blood pressure which appeared to be both prompt and permanent. Two typical tracings are shown in Figs. 6 and 7. These results seemed very striking and further experiments are in progress.

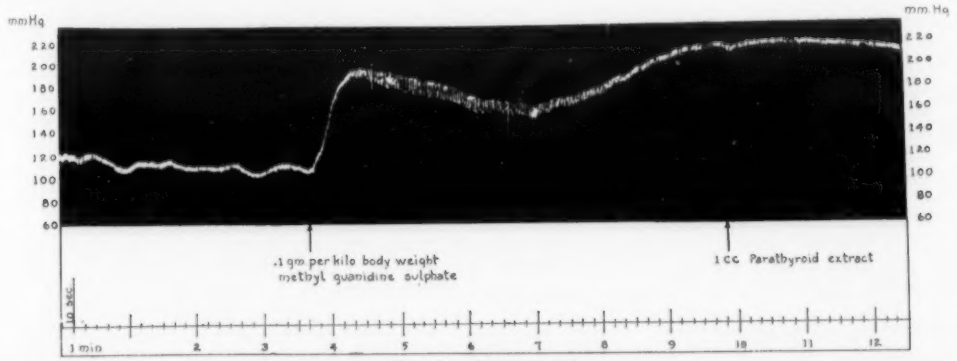

FIG. 3. (First part)

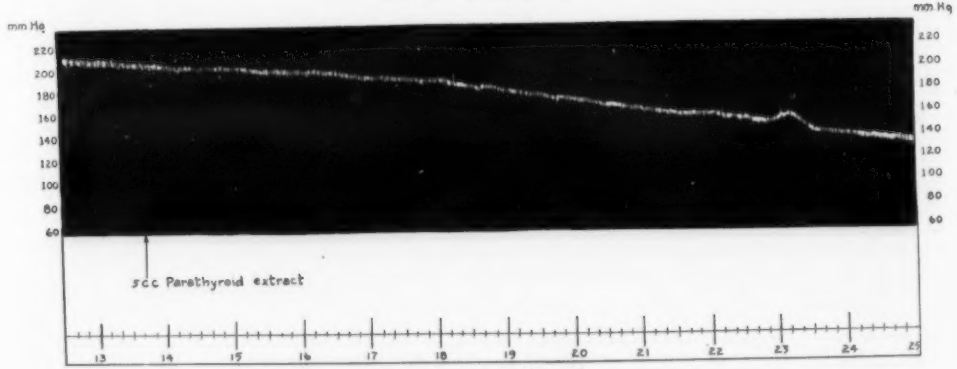

FIG. 3. (Second part)

FIG. 3. FAILURE OF ATROPINE TO INHIBIT DEPRESSOR EFFECT OF LIVER EXTRACT UPON HYPERTENSION PRODUCED BY METHYL GUANIDINE SULPHATE

Intravenous injections

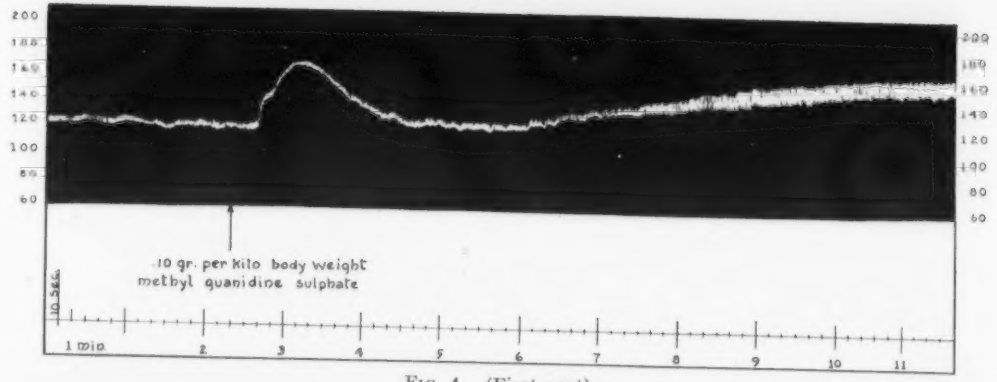

FIG. 4. (First part)

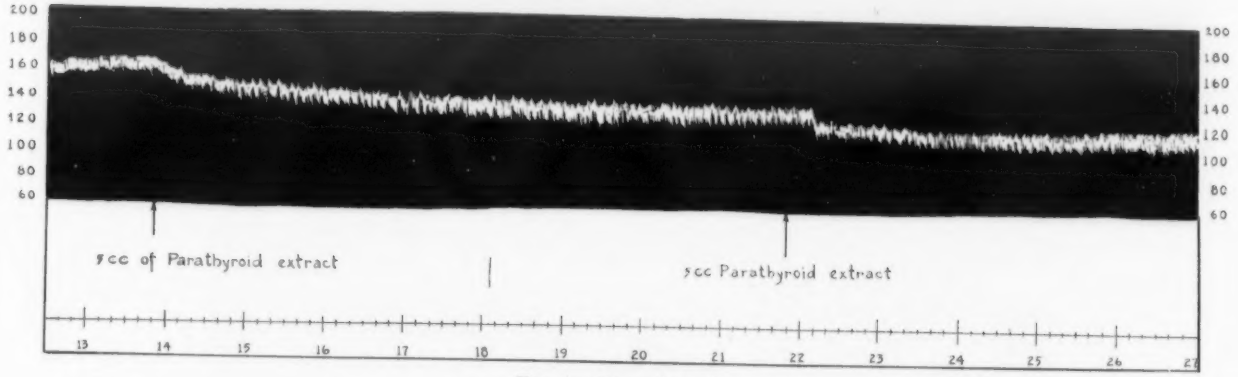

FIG. 4. (Second part)

FIG. 4. EFFECT OF LIVER EXTRACT UPON ELEVATION OF BLOOD PRESSURE CAUSED BY METHYL GUANIDINE SULPHATE
Note gradual fall in blood pressure. Intravenous injections of liver extract and of methyl guanidine sulphate.

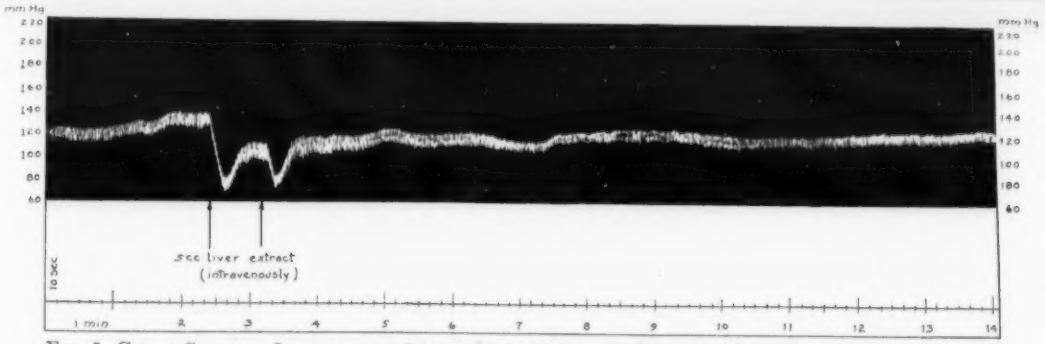

FIG. 5. CURVE SHOWING INTRAVENOUS INJECTIONS OF LIVER EXTRACT TO HAVE NO CONSTANT EFFECT UPON THE NORMAL BLOOD PRESSURE OF A DOG

The coagulation time of the blood was determined five times during the course of the experiment. (Method of Lee and White.) The coagulation time remained three minutes.

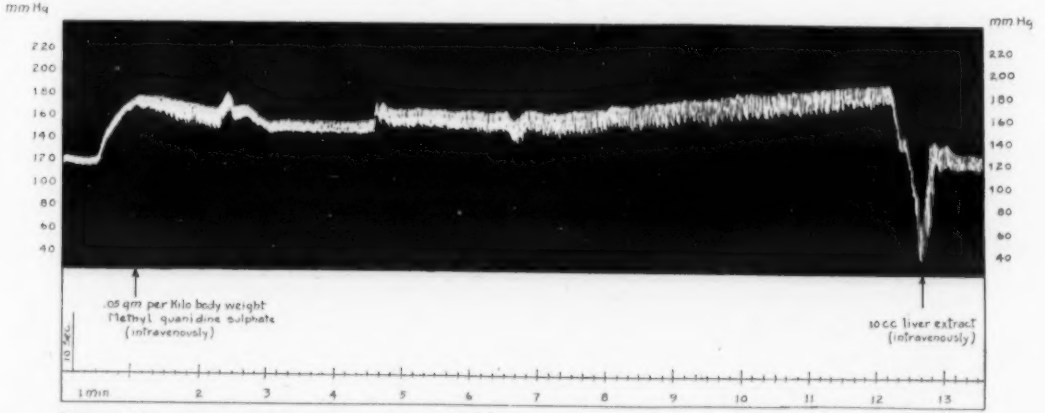

FIG. 6. CURVE SHOWING EFFECT OF LIVER EXTRACT UPON THE HYPERTENSION PRODUCED BY METHYL GUANIDINE SULPHATE

Blood pressure lowered and continued so for one hour

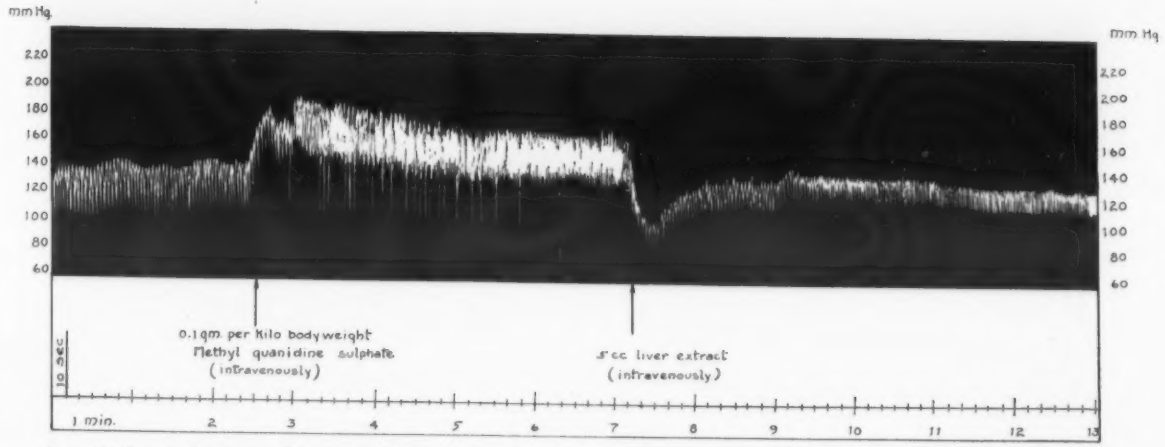

FIG. 7. CURVE SHOWING EFFECT OF LIVER EXTRACT UPON THE HYPERTENSION PRODUCED BY METHYL GUANIDINE SULPHATE

Blood pressure lowered and continued so for two hours

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Gonorrheal Endocervicitis Treated with Strong Solutions of Mercurochrome

By Leo Brady

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THE care of patients with endocervicitis has always seemed to me to be unsatisfactory not only because of the obstinacy of the condition, but also because of the difficulty with which the results of treatment can be judged. The very multiplicity of remedies used indicates that none has proved entirely satisfactory. Some men use iodine, others argyrol, others silver nitrate, while still others, more radical, apply pure carbolic acid to the cervix.

The fact that such a large proportion of these patients are eventually operated on indicates that the usual medical procedures prove unsuccessful in a large percentage of cases. Although in the present paper I intend to speak more of the medical than of the surgical treatment of endocervicitis, it may not be out of place to digress for a moment in order to emphasize the fact that to cure patients whose leucorrhea is due to a laceration of the cervix rather than to an infection of the endocervix, an operation is almost always necessary and that, consequently, to subject them to a long series of cervical treatments is useless and expensive. For these patients a trachelorrhaphy, and not amputation of the cervix, is the operation of choice, unless they are beyond the childbearing age, for most women whose cervices have been amputated, when they become pregnant, either abort or have trouble at the time of labor. When we are forced, through the failure of our medical treatments, to operate in cases of endocervicitis not complicated by a laceration of the cervix, the best operation is probably a Sturmdorf tracheloplasty. By this procedure, we remove a large part of the infected gland-bearing portion of the cervix, thus curing the leucorrhea, and, as we shorten the cervix to only a very slight extent, no complications are apt to occur in future pregnancies. Radical cauterization of the cervix, first advocated by Guy Hunner (1), should be here mentioned as a method of distinct value, as it often greatly lessens the discharge, is simple in technique and generally can be done without an anaesthetic. Corbus and O'Connor (2), in an article on the treatment of gonorrheal endocervicitis (*Surgery, Gynecology and Obstetrics*, January, 1924), report some very good results obtained by using a special cervical thermophore which they have devised, but at the same time they say: "The method is contraindicated in the early acute stages of the infection or when evident active pelvic inflammatory changes such as

salpingitis or pelvic cellulitis are present."

The real purpose of this paper, however, is not so much to discuss the whole subject of endocervicitis as to give a preliminary report of the results obtained by a method of treatment carried out at the House of the Good Shepherd on women infected with gonorrhea. When, fifteen months ago, under the State Department of Health, I took charge of this work, it was at once evident to me that I had there an unusually good opportunity of making a thorough study of gonorrhea in women. For as these women are usually committed to the institution for a period of one year, at least, they are compelled to submit to the prescribed treatments and there is no chance of a new infection.

Two problems at once presented themselves, the first was to develop a method by means of which we could be sure when gonorrheal endocervicitis is really cured, the second, to decide on what remedies should be used. In deciding these problems, we endeavored to bear in mind the involved anatomical and pathological conditions which briefly are as follows:

In women gonorrheal infection is primary in one of three localities: the urethra, Bartholin glands or the endocervix. Fortunately, the lesions in the urethra usually heal spontaneously and local treatments are seldom necessary. In our series only three patients required treatment for urethritis and in them injections of a mild antiseptic solution quickly relieved the condition. When Bartholin's glands are infected, it is best to remove these structures, but to quote from Shutter (3), "The incidence of infection in the glands of Bartholin is estimated at from 15 to 35 per cent of chronically infected cases." Without going into a discussion as to how frequently the infection is primary in the endocervix and how frequently, when in this locality, it is secondary to one in the urethra, the condition which we found to be present in our patients was an endocervicitis and it was toward the cure of this condition that our efforts were principally directed.

It is only in the young child, when the cornified layer of the epithelium has not yet formed, or in the aged, when this layer has disappeared, that the vagina is involved in Neisser infections. For this reason strong vaginal douches are not only unnecessary but are absolutely harmful in the treatment of gonorrhea in adult women, for they not only do not reach the endocervix but, if they are strong enough to break down the cornified layer of the vagina, they provide a path by which the gonococci can enter tissues from which, under ordinary conditions, they are excluded by a natural barrier.

At the beginning of our work at the House of the Good Shepherd, it was decided that each patient with gonorrhea should have a thorough gynecological examination, including the taking of smears from the urethra and cervix; that treatment should be at once started and that smears should be taken at regular intervals of two weeks. For simplicity of records, the amount of discharge was recorded by numbers (*0* = absolutely no discharge, *1* = about the amount that is normal in a great many women just before the onset of menstruation,

2 = a moderate and *3* = a profuse leucorrhea). The following tests were decided upon as being necessary before a patient could be considered cured. She must have had three successive negative smears taken at two week intervals on days on which the vaginal douches were omitted, be then released from all treatment for a period of one month, and have a negative smear at the end of this time and not over what we called a *1* discharge. In the vast majority of our cured cases, there was absolutely no discharge at the end of this month. In some clinics, three negative smears taken in succession are regarded as representing a cure. We have added the fourth negative smear taken after a month without treatment as an extra safeguard and feel after a patient has met these requirements that she is really cured. By taking the three negative smears at intervals of two weeks, one at least of these was sure to come either just before or after the menstrual periods when organisms can be found which are not demonstrable at other times. Glingar (4), in a recent article from the Maria-Theresia Frauenhospital at Vienna, emphasizes the value in diagnosing gonorrhea in women of taking smears immediately after the menstrual period.

In the last few weeks, we have re-examined two patients discharged as cured five months, two discharged six months, two seven months, and two eight months previously. As all of these patients were found to have no vaginal discharge and had negative smears, we are encouraged in our belief that the standards we have set for a cure are sufficiently rigid.

At the beginning of this work we adopted a definite method of therapy which we have now carried out for fifteen months. Vaginal douches are given daily except during the menstrual period. A powder containing boric acid, carbolic acid and menthol, not unlike the antiseptic powder in the National Formulary is used in the douches, but probably ordinary salt would be just as efficacious. Not that by this I mean to say that douches never do good—the heat in hot douches is of real curative value in salpingitis and pelvic cellulitis, but in the treatment of uncomplicated endocervicitis all that douches accomplish is to carry away the discharge that is in the vagina.

We decided upon mercurochrome as the drug which we would apply to the cervix, after taking into consideration the following facts: The mucous membrane of the endocervix is composed of complicated racemose glands with small ducts emptying into the cervical canal and is thrown into definite folds rotating from a central line, an arrangement commonly called the arbor vitae. It is this structural arrangement—folds with the deep recesses between them—that makes the endocervix such a favorable soil for the gonococcus. Whether, as is generally believed, it is in the ducts of the cervical glands that the infection persists or whether, as Kennedy (5) has recently suggested, it is in the interstitial tissue between the glands, certainly it is in the endocervix and not in the vaginal portion of the cervix that the disease is most resistant. In order to reach the gonococci which bury themselves deep down between the folds of the

cervical mucosa, a drug seemed needed which had a strong capacity not only for antisepsis, but also for penetration, and it was because mercurochrome, as shown by Young and White (6), possesses this latter power to such a marked degree that we decided on this drug. We have not been content to paint merely the vaginal portion of the cervix with our solution, but have applied it along the entire endocervix. By using a small wire applicator and getting good exposure, we have been able, in practically all of the cases, to reach, with mercurochrome, all the way to the internal os. We have made no attempt to treat the endometrium, for it is well known that the body of the uterus possesses considerable natural immunity to gonorrheal infections.

Mercurochrome is used for kidney lavage in a 1 per cent solution, for the urethra, in strengths varying from 0.1 to 5 per cent, and as a general antiseptic, to compete with tincture of iodine, in a 2 per cent solution. As it is possible and indeed recommended by some men to use strong silver nitrate solutions or pure carbolic acid in the cervix in the treatment of endocervicitis, and as the use of these solutions cause the patient no pain because of the insensitiveness of the cervix, it seemed to me that it would be safe to employ a very concentrated solution of mercurochrome so as to utilize to the fullest extent its power of penetration. Rupel (7) of Indianapolis has recently recommended the use of a 6 per cent solution of mercurochrome in early gonorrheal infection in men, and von Lackum and Hagar (8), writing from the Mayo Clinic, state that in the treatment of gonorrhea in women, they have had excellent results from its use, alternated with silver compound and iodine. The two latter authors paint the vault of the vagina with 1 per cent mercurochrome and place pure crystals in the external os of the cervix, but as the mercurochrome therapy is apparently carried on for only two weeks, and no figures are given of the results obtained nor any account of the criteria used in judging whether or not the condition was entirely cured, it is difficult to determine from their article what was accomplished by the use of mercurochrome. I have in a rather careful search of the literature come across no other mention of solutions of mercurochrome being used clinically in strengths of over 5 per cent.

In the work at the House of the Good Shepherd a 20 per cent solution of mercurochrome has been used and our experience has shown that, for the endocervix, this drug is absolutely safe in this strength. There has never been any evidence of general toxic symptoms developing nor of any local destruction of tissue. We have painted a series of normal cervices with iodine, another with 10 per cent silver nitrate and a third series with 20 per cent mercurochrome and two days later re-examined all these patients. Less local reaction followed the use of the mercurochrome than after the other two drugs. Pure carbolic acid, of course, causes much more local destruction of tissue than any of these drugs.

Briefly, our method of treatment has been as follows: The patient received douches daily except during the menstrual periods and on the day

on which smears were to be taken. Twice a week she was placed in the lithotomy position and the cervix well exposed with a bi-valve duck-bill speculum. The amount of discharge coming from the external os was recorded by numbers, as previously mentioned, and the discharge then carefully removed. The entire vaginal portion of the cervix was well painted with 20 per cent mercurochrome and then a small amount of cotton on a thin wire applicator well soaked in this solution was introduced into the external os and carried through the cervical canal to the internal os. Occasionally, the applicator would slide so easily through the cervix that it would impinge on the fundus uteri. I have never seen any harm result from this, although we prefer that it should not happen. No glycerin tampons were used, in order that we might be able better to interpret the results obtained and that the dehydrating influence of the glycerin might not interfere with the penetration of the mercurochrome solution into the deeper tissues.

At the end of fifteen months the following report of our work is presented: Thirty-six patients with endocervicitis have been treated. The age of the oldest girl was 20 years, that of the two youngest 7 and 10. The ages of the other 33 patients ranged from 13 to 19 years. In all except the two patients 7 and 10 years of age, there was a history of coitus and the hymen, on examination, was found to be ruptured. These two children were, of course, examined only through the rectum and were treated by vaginal instillations given in the knee-chest position. The 20 per cent mercurochrome in these two patients did not irritate the vagina and the condition cleared up promptly. One four-year-old child, whom I treated at my office, complained of some burning after the use of the 20 per cent solution, but no local irritation was produced and the gonorrheal infection cleared up promptly. As in children under ten years of age, the infection is in the vagina and not in the endocervix, probably the 5 per cent solution of mercurochrome would be just as effective for them.

On pelvic examination, thickening of Bartholin's glands was found in two instances and in eight evidence of salpingitis was present.

In 32 out of 36 patients typical Gram-negative intracellular diplococci were found; in four, although the history and the presence of a profuse leucorrheal discharge made us fairly certain of our diagnosis, the organisms could never be demonstrated.

Of the 32 proven cases, in 22 instances the first smear was positive, in 9 the second. The remaining case was of unusual interest. The patient had been treated for five months for a profuse leucorrheal discharge before the mercurochrome treatment was started. Iodine and glycerin tampons had been used and then pure carbolic acid, but the discharge had not lessened. Not, however, until the end of the fifth month was a positive smear obtained. At this point, the mercurochrome treatments were started and at the end of two months the discharge had completely disappeared and by the end of three months the patient had had her four negative smears. This may mean that the carbolic acid and the iodine had

destroyed the organisms superficially situated, but had not reached those situated in the deep tissue around the ducts of the cervical glands. It is not impossible that a drug like pure carbolic acid which causes such a tremendous superficial necrosis of tissue might secondarily produce such a great amount of scar tissue that it would be difficult in the future to get through this scar tissue to the deeply situated gonococci or to obtain positive smears showing the presence of the organisms.

In this connection two other cases might be mentioned. Both of these patients had been treated with other remedies before mercurochrome was started. The one had had nine months of silver nitrate and glycerin tampons and then five months of iodine and glycerin tampons. At the end of this time two negative smears were obtained. She then was submitted to two weeks' treatment with mercurochrome during which the third smear was obtained, and was then released from treatment for one month. For the other patient carbolic acid and glycerin tampons were employed for two months with two negative smears and then two weeks of mercurochrome treatment during which the third negative smear was obtained. Both of these patients at the end of their month without treatment returned with a considerable amount of discharge and positive smears. There have been in our series no instance in which the patient, after the entire treatment has been carried out with mercurochrome and three negative smears obtained at two-week intervals, has returned with positive smears at the end of a month without treatment.

SUMMARY

In the 4 patients who had a profuse leucorrhea, but whose smears were never positive, the discharge cleared up entirely in six weeks, and after a month without treatment, on examination, no discharge was seen to be present and the vaginal smears were still negative. In the 32 patients, with positive smears, our treatment absolutely cured in 25, probably cured in 4 more and failed in 3 cases. The reason we claim only a probable cure in these 4 instances is that in them we were unable to apply the entire series of our tests that we stipulated as necessary to prove a cure, because these 4 patients were released by the court when they had shown three negative smears and were waiting their month before the final examination. As all four of these patients had had three negative smears in succession and when released by the court had no vaginal discharge, according to the standards taken by many, we might claim a cure in 29 out of 32 cases. The three patients on whom the treatment failed all had, on admission, a history suggesting salpingitis and definite evidence of tubal involvement, and in two out of the three there was an infection of Bartholin's glands. After conservative treatment for many months with no result, I finally operated on them. The other five patients with evidence of salpingitis on admission cleared up entirely under hot douches and mercurochrome treatments. A point that should be emphasized is that there has not been a single instance of the development of a salpingitis in a patient while under this treatment.

This fact is in marked contrast to

what Shutter (3) says in a recent article in which he advocates the rather radical use of the cautery in the treatment of chronic gonorrhea in the female. To quote from that article: "Because of the danger of extension, treatment has seemed inadvisable for two or three days before and after menstruation. Though tubal involvement has occurred two or three times after cervical cauterization in no instance did it necessitate abdominal section." It has seemed to us that just before and just after the menstural period are especially good times, not only, as has been said, for making the diagnosis, but also in which to carry out our treatments, because the increased vascularity at that time probably brings to the surface organisms which in the interim would be hidden deep down in the folds of the mucosa.

In the 23 cases for which an absolute cure is claimed, the average time the patient was under our care, including the month in which all cervical treatments were suspended, was $3\frac{2}{3}$ months.

Grouping together the 24 cases of absolute cure and the 5 cases of probably cure, in these 29 cases in 14 instances the smear taken at the end of two weeks' therapy was negative and gonococci were never found in the discharge after that time. In 7 other instances the third smear taken, that is, the one after one month's treatment, was negative and no further positive smear was obtained. Four patients gave a negative smear at the end of two months and three others at the end of ten weeks. One patient required four months to cure.

Without running control series of cases, we do not feel justified in claiming any superiority of this over other methods in the treatment of endocervicitis; nevertheless, we do feel that it might be of interest to give briefly the histories of a few patients who had been treated at the House of the Good Shepherd with other remedies before the mercurochrome therapy was started. These cases are not presented to suggest how unsatisfactory other methods of treatment have been, but merely to point out how effective strong solutions of mercurochrome have been in cases that have been markedly resistent to other drugs.

Case 106. A. W. Aged 17. Treated 14 months with 10 per cent silver nitrate and glycerin tampons; then one month with iodine and tampons; then four months with carbolic acid and tampons. At the end of this time a profuse discharge was still present and gonococci were found in it. At this point mercurochrome was started. No further positive smears were obtained; the patient was discharged in three months, with no leucorrhoea. Re-examined at end of eight months. No discharge present. Smear negative.

Case 256. M. S. Aged 16. Iodine and tampons for six months. Discharge still *3*. Cauterized with cautery. Discharge dropped to *2*. Gonococci still present. Mercurochrome started. No further positive smears. Discharged in four months with no leucorrhoea.

Case 260. L. A. Aged 16. Treated with iodine and glycerin tampons for three months. Carbolic acid and glycerin for three months. Smear positive. Discharge *3*. Mercurochrome started. No further positive smears. Discharged in four months without leucorrhoea. Re-examined at end of eight months. No discharge present. Smear negative.

Case 262. A. D. Aged 16. Treated three months with iodine and glycerin tampons. Discharge still *3*, although no gonococci were present at the end of three months. Mercurochrome started. No further positive smear. Discharged in two and a half months with no leucorrhoea.

Case 274. E. S. Aged 15. Treated two two months with argyrol and tampons. Discharge still *3*, and gonococci present in the smear. Mercurochrome started. Smears never again positive. Discharged in four months. Re-examined at end 5 months. No discharge present. Smear negative.

Case 279. M. P. Aged 20. Treated two months with iodine and glycerin. Smear still positive. Discharge *3*. Mercurochrome started. Smear taken at the end of two weeks, positive. No further positive smears after this. Discharged in two and a half months with no leucorrhoea.

Case 282. R. A. Aged 16. Treated two months with iodine and glycerin tampons. Smear still positive and discharge *3*. Mercurochrome started. Smear positive at the end of two weeks. No further positive smear. Discharged in four months. Re-examined at end of two months. No discharge present. Smear negative.

Case 285. R. S. Aged 16. Treated two months with carbolic acid and glycerin tampons. One month with iodine and tampons. Smear still positive. Discharge *2*. Mercurochrome started. No further positive smears obtained. Discharged in four and a half months.

Case 269A. Aged 10. Unruptured hymen. Treated two months with vaginal instillations of 10 per cent argyrol. Discharge still *2*, and gonococci present in it. Mercurochrome treatment started. No further positive smear. Discharged with no luecorrhoea in two and a half months.

Case 270. S. B. Aged 7. Unruptured hymen. Treated six months with vaginal instillations of 10 per cent argyrol. Discharge still *2* and gonococci present. Mercurochrome treatment started. No further positive smears. Discharged with no leucorrhoea in two and a half months. Re-examined in six months. No discharge present. Smear negative.

We present this method of treating endocervicitis which, judged by standards that we feel to have been rigid, has in our series produced a real cure in all cases of gonorrheal endocervicitis uncomplicated by salpingitis or Bartholinitis. We feel that infection of Bartholin glands should be treated by their complete removal. Two distinct advantages of this method are the simplicity of technique and the minimal danger of a resulting salpingitis.

In conclusion, I wish to extend my thanks to the Maryland State Department of Health for the support which they have given me in carrying out this study, and to Dr. Thomas S. Cullen and Dr. Hugh Young for their suggestions and criticisms in the preparation of this paper.

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On the Preparation of Haematoporphyrin from Carbon Monoxide Haemoglobin

By Hans Smetana

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In 1912, Arnold (1) reported a method of extracting haematoporphyrin from carbon monoxide haemoglobin, as a modification of that of Hoppe Seyler. His directions are as follows:

Fresh defibrinated blood is saturated with carbon monoxide (or ordinary illuminating gas). The blood is then transferred, little by little, into concentrated H_2SO_4, the acid being constantly stirred. This process is continued until drops of this mixture will form an apparently dense clot when poured into water. The mixture is now poured into a large quantity of distilled water, which causes precipitation of the haematoporphyrin and also of the proteins. The amorphous precipitate is then collected in a filter and quickly washed with distilled water several times. Arnold went no further and published no studies of the haematoporphyrin thus produced.

This method appeared so simple that it seemed worth while to study the properties of the finished product and to compare it with the haematoporphyrin obtained by the well-known Nencki method (2). With this idea, haematoporphyrin was prepared by the above-outlined Arnold method, and tests yielded the following observations:

The substance gives the typical acid haematoporphyrin spectrum when dissolved in 25 per cent hydrochloric acid, whereas its solution in n/10 sodium hydroxide does not show the spectrum of alkaline haematoporphyrin clearly.

If a mouse, injected with a saturated solution of this substance in n/10 sodium hydroxide, is exposed to sunlight, the animal shows only slight irritation and does not develop the severe haematoporphyrin shock.

Crystals of haematoporphyrin hydrochloride were obtained from this raw material by the following procedure:

The amorphous haematoporphyrin precipitate on the filter is thoroughly washed with water until the washings become neutral. The residuum is dissolved in 1 per cent hydrochloric acid and this solution is filtered. One-tenth volume of concentrated hydrochloric acid is added, forming a voluminous precipitate. After filtration, a clear, diluted, purplish-red solution is obtained, which shows the acid haematoporphyrin spectrum very clearly. This solution is now evaporated *in vacuo* or in a current of air, without the use of heat, and rhomboid needles are formed which are radially arranged. The solution of these crystals in n/10 sodium hydroxide gives

the typical alkaline haematoporphyrin spectrum.

An animal injected with a concentrated solution of these crystals in n/10 sodium hydroxide develops the typical signs of haematoporphyrin shock after exposure to sunlight.

The amorphous precipitate which is formed after pouring the sulphuric acid-haematoporphyrin mixture into distilled water, can be partly dissolved in alcohol, after having been washed with water. Six to ten or more volumes of ether are added and the solution is then filtered. The alcohol is washed away with a current of water through a separatory funnel. The ether is then separated from the water and filtered free from the precipitated calcium salt of haematoporphyrin. After concentration of the ether over the water-bath, the spectrum of this ether solution of haematoporphyrin shows the following absorption bands, which correspond exactly to those described by Willstätter and Fischer (2), who studied the ether spectrum of haematoporphyrin obtained by Nencki's preparation (Fig. 1).

I	625	A dark line
II	600	A light shadow
III	570–590	A shadow
	570–580	A dark band
IV	530–540	A dark band
V	485–514	A dark band
	Absorption in the ultra-violet from 400 to about 440	

This ether solution of haematoporphyrin is not stable; precipitation may occur and the spectrum also changes, now showing two bands in red at 655–660 and 625–630, while the other bands are unchanged.

The haematoporphyrin-hydrochloride can be obtained from this ether solution of haematoporphyrin by means of repeated shakings with 10 per cent HCl in a separatory funnel. Almost all the haematoporphyrin goes into the hydrochloric acid solution and very little is left in the ether. As one looks through the spectroscope at the separatory funnel containing the ether and hydrochloride solution of haematoporphyrin, the spectrum changes from the ether spectrum to the hydrochloric acid spectrum, according to the different level. After the haematoporphyrin-hydrochloride solution has been separated from the ether, the fluid is heated (to not over 70°) in

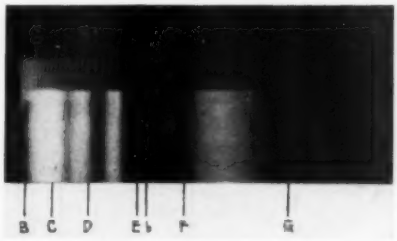

Fig. 1

order to evaporate the ether which is dissolved in the acid. After this, the solution can be evaporated without using heat, and rhomboid crystals are formed besides the amorphous haematoporphyrin-chloride.

If the solution of haematoporphyrin in concentrated sulphuric acid is poured into alcohol, the haematoporphyrin is not precipitated but goes readily into solution. From this solution it may be precipitated with sodium hydroxide and sodium acetate.

It was found that the haematoporphyrin obtained in this manner is much purer than the haematoporphyrin precipitated in distilled water.

The modified procedure is as follows:

Saturate fresh defibrinated blood thoroughly with carbon monoxide. This process takes about three hours for a liter of blood, when the ordinary illuminating gas is used. Pour the blood, little by little, into concentrated sulphuric acid, which is kept constantly stirred. About 1 liter of carbon monoxide blood is transferred into 1 liter of concentrated sulphuric acid. The haematoporphyrin, formed by the acid, gives the solution a bright raspberry red color. This differs entirely from the brownish color

FIG. 2

of haemin which may be formed when the blood is not completely saturated with carbon monoxide or when too much blood is poured at once into concentrated sulphuric acid.

Pour one liter of this mixture into three liters of 95 per cent alcohol. Eight liters of alcohol are then added, making the proportion of alcohol about 85 per cent. Almost all the proteins are precipitated after standing for several hours. The solution is then filtered.

Precipitate the haematoporphyrin in the filtrate, by adding a mixture of 2000 cc. of saturated sodium acetate and 200 cc. of sodium hydroxide to 1000 cc. of the acid alcohol. The supernatant fluid should be almost colorless.

Collect the brownish precipitate in a large filter and wash with running warm water for several hours, in order to dissolve the crystals of the various salts, formed by the sulphuric acid, sodium hydroxide and sodium acetate.

Dissolve the precipitate in 1.0 to 1.5 per cent hydrochloric acid by means of heat (not over 70°C.) and filter. A clear, purplish-red solution is obtained, which shows the spectrum of the acid haematoporphyrin very clearly.

Add 1.10 volumes of concentrated hydrochloric acid and filter, if an amorphous precipitate is formed.

Evaporate the fluid without using heat. Brownish crystals of hydrochloric acid haematoporphyrin are formed having the shape of rhomboid needles which are radially arranged (Fig. 2).

The crystals are dried *in vacuo* over sulphuric acid and soda lime. By grinding them, a red powder is obtained. This substance is readily soluble in dilute hydrochloric acid, in n/10 sodium hydroxide and fairly soluble in alcohol; it is almost insoluble in ether.

When it is dissolved in 25 per cent hydrochloric acid, the spectrum shows the following absorption bands:

I	588–598
II	541–559
	Absorption of the violet end

When it is dissolved in n/10 sodium

hydroxide, the bands are located as follows:

I	612–622
II	560–578
III	535–545
IV	495–512

Absorption of the violet end of the spectrum

Mice, subcutaneously injected with 1 cc. of a 1 per cent solution of the powder in n/10 sodium hydroxide and exposed to sunlight—after having been kept in the dark for one hour in order to absorb the injected haematoporphyrin—develop the typical signs of the haematoporphyrin shock almost immediately after the exposure; they are restless and scratch the nose, ears and body continuously. Later on their legs—especially the hind legs—become paretic and finally the animals die. Death occurs usually in from one to three hours after exposure. Sometimes the mice do not die during the exposure, but some hours afterwards. Control animals, injected with 1, 1.5 and 2 cc. of a 1 per cent haematoporphyrin solution in n/10 sodium hydroxide and kept in the dark for longer than a week, did not shown any reaction whatever.

The amorphous haematoporphyrin precipitate formed after adding sodium acetate plus sodium hydroxide to the acid alcoholic haematoporphyrin solution can be partly dissolved in alcohol. Five to ten or more volumes of ether are added and after filtration the ether solution of haematoporphyrin may be obtained in the manner described above. After being dried with anhydrous sodium sulphate, the ether may be concentrated to less than one-tenth of the volume over the water-bath.

The deep red solution is allowed to evaporate at room temperature. There are small, well rounded, brownish-red leaflets seen among the amorphous masses of haematoporphyrin.

From the ether solution the haematoporphyrin-hydrochloride may be obtained again by shaking with 10 per cent hydrochloric acid, and crystals of haematoporphyrin hydrochloride formed in the manner described above. The acid or alkaline spectrum of this substance is very pure. It causes the haematoporphyrin shock in mice exposed to sunlight.

SUMMARY

A modification of the Arnold method for the preparation of haematoporphyrin from carbon monoxide haemoglobin is proposed. By this method comparatively pure haematoporphyrin preparations can be easily obtained.

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A Bacillus of the Paratyphoid Group

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In a previous paper (1) the author reported a Gram-negative bacillus as the case of spontaneous infectious otitis so common in colonies of albino rats. By the usual cultural methods this organism seemed to be identical with B. paratyphoid B. As it was not agglutinated by immune serum of several strains of the latter, including one of animal origin, a more complete bacteriological study was undertaken to determine the relation of this bacillus to the human paratyphoids, as well as those of animal origin.

The bacillus to be described, and for which I propose the name *Salmonella muriotitis*, was isolated from the purulent material invariably found in the middle ear of animals suffering from suppurative otitis. Six cultures from infected animals obtained from widely separated regions have been studied. Strains 1 and 2 were isolated from 'healthy' stock purchased from a breeding station in Philadelphia. Two others, 3 and 4, from members of a colony of 1500 (bred from Wistar stock) at Buffalo, N. Y. Strain 3 was from an animal with symptoms of disequilibration and 4 from one which at autopsy showed middle ear involvement only. Strains 5 and 6 are from members of a Baltimore colony, the former from an animal without symptoms, the latter from one with right internal ear involvement.

Together with these, cultures of paratyphoids from as many different sources as could be obtained, were studied for comparison. Two of these were human strains. One, paratyphoid B 224 N. Y.; the other, B. enteritidis. The remaining four were paratyphoids of animal origin from different species. B. pestis caviae 146 from a guinea-pig and B. pestis caviae E. and C. 6 Reg. 3, of rodent origin (wild rat), the causative organism of an epidemic in New York (2), due to infected pastry. Both of these were obtained from Dr. C. Krumwiede of the Department of Health, New York City. A culture of B. typhi murium, isolated by N. J. Howard from spontaneously infected mice in this laboratory, and one each of hog cholera and B. abortus equi, were included.

MORPHOLOGY

The examination of smears from 24-hour agar cultures shows a short bacillus with rounded ends (Fig. 1). The length varies from 1 to 2 micra, and the average thickness is about 0.4 micron. When grown in broth, it is slightly thicker, averaging about 0.5 micron. Smears of ear pus often

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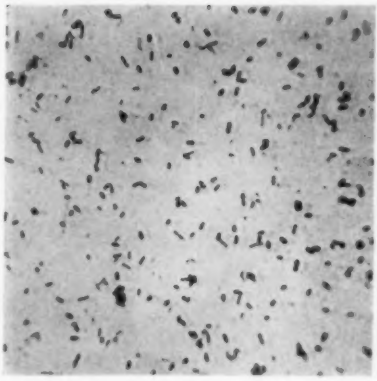

FIG. 1. SMEAR FROM 24-HOUR GROWTH ON AGAR

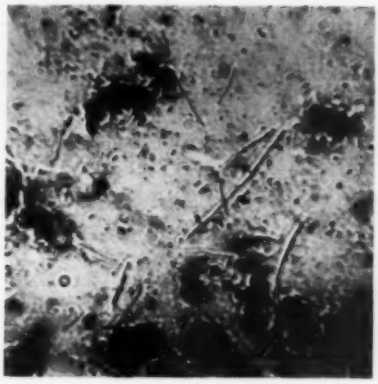

FIG. 2. SMEAR FROM EAR PUS SHOWING FILAMENTOUS FORMS

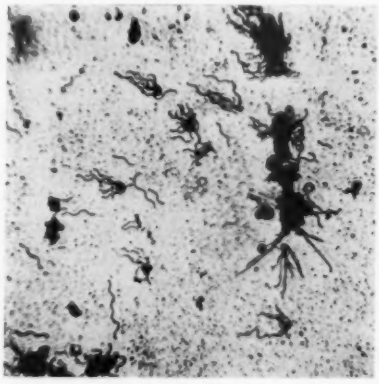

FIG. 3. FLAGELLA STAIN OF 18-HOUR AGAR CULTURE

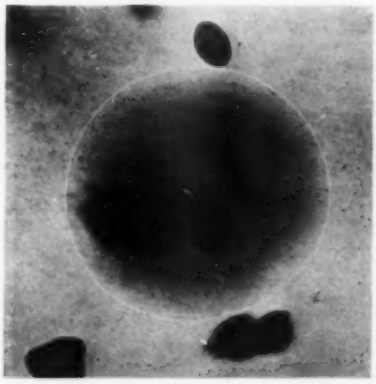

FIG. 4. SUPERFICIAL AND DEEP COLONIES FROM GELATIN PLATE CULTURE, 3-DAY GROWTH AT ROOM TEMPERATURE

show long, filamentous forms (Fig. 2). This pleomorphism is also observed at times in cultures.

In hanging-drop preparations it is sluggishly motile. Numerous flagella are visible by dark field illumination and stained specimens reveal from 6 to 8 peripherally arranged (Fig. 3).

The organism is Gram-negative and strains readily with all the ordinary anilin dyes. No spores or capsule could be demonstrated. It is not acid-fast.

CULTURAL CHARACTERISTICS

On agar slants the growth is visible in 3 or 4 hours as a thin, semi-transparent, gray surface film. After 24 hours it has become more opaque, with well defined margins but presents no distinctive features. The growth is more abundant on agar-containing glucose.

On agar plates the surface colonies appear as small discs—white by reflected light and gray-blue by trans-

mitted light. They are quite transparent when examined with a low power objective even with the substage diaphragm stopped down so as to admit only a minimal amount of light; in this respect resembling the dew-drop appearance of B. influenzae. They are finely granular and have a sharp, well defined border. Their average size is about 0.5 mm.

In gelatin plates the superficial colonies are also delicate, semi-transparent discs, with sharp even border. The deep colonies are much smaller, round or olive-shaped and more opaque than the superficial ones. The growth is well developed by the third day (Fig. 4).

In gelatin stab cultures growth occurs upon the surface and along the puncture tract, producing a nail-like growth. There is no gas formed and the medium is not liquefied even after two months' growth.

On potato having an alkaline reaction growth is abundant. It becomes visible in about 48 hours as a faint gray, glistening layer which later turns a straw yellow, and, after 8 or 10 days, a light brown color.

In milk there is a distinct alkaline reaction at the end of from 3 to 5 days, without coagulation.

In plain bouillon there is a uniform turbidity at the end of 24 hours, of about the density of that produced by the typhoid bacillus.

There is no hemolysis in blood agar plates. Loeffler's blood serum is not liquefied.

In Dunham's peptone solution the growth is slow. Turbidity appears in about 36 hours. The pellicle is not marked. Indol is not formed. Nitrates are not reduced.

In lead acetate peptone agar a brown color is produced, indicating the formation of hydrogen sulphide.

CARBOHYDRATE FERMENTATION

The fermentation reactions of S. muriotitis on the various carbohydrates are given in Table II, which also contains those of the other organisms studied for comparison. Fermented infusion or extract bouillon containing 1 per cent of the sugar, with bromcresol purple, or Andrade indicator, was used. Gas was determined in Durham fermentation tubes and the Smith tube, but, as it was found to be quite variable with both types, the method of overlaying the broth with a layer of vaseline, as described by Brown (3), was used and the amount of gas measured as volumes of the amount of medium. The gas production of muriotitis is worthy of note. Xylose is the only sugar in which an appreciable amount of gas is produced. No gas is formed in sorbite, while the amount produced, in this carbohydrate, by all the other organisms studied is large.

Analysis of the gas from dextrose, galactose and xylose gives an average gas ratio $\frac{(H_2)}{(CO_2)}$ of $\frac{30}{70}$.

Inosite is not fermented, but this is not a distinguishing feature, as positive and negative inosite fermenters have been reported (4). Rhamnose is never fermented until the third day, while all the other strains produce unquestionable fermentation in 12 hours. This low avidity for rhamnose has been observed in all freshly isolated strains, and has persisted in the seventy-fourth generation of a culture 8 months old, and after several transmissions

TABLE I

Carbohydrate fermentation reactions of muriotitis and other paratyphoids studied

ORGANISM	ADONITE	ARABINOSE	DEXTRIN	DEXTROSE	DULCITE	GALACTOSE	GLYCEROL	INOSITE	INULIN	LACTOSE	LEVULOSE	MANNITE	MALTOSE	RAFFINOSE	RHAMNOSE	SACCHAROSE	SALICIN	SORBITE	TREHALOSE	XYLOSE
S. muriotitis, strains 1, 2, 3, 4, 5, and 6	–	A 0.1*	–	A 0.1	A 0.1	A 0.1	–	–	–	–	A 0	A 0.1	A 0.1	–	D 0	–	–	A 0	A 0.1	A 0.4
B. paratyphoid B, human	–	A 0.2	–	A 0.4	A 2.1	A 0.5	–	A 0.2	–	–	A 0.5	A 0.8	A 0.8	–	A 0.1	–	–	A 1.2		A 0
B. enteritidis	–	A	–	A	A	A	–	–	–	–	A	A	A	–	A	–	–	A		A
B. pestis caviae, 146	–	A 0.1	–	A 0.4	A 0.7	A 0.4	–	–	–	–	A 0.2	A 0.5	A 0.6	–	A 0.4	–	–	A 0.8		A 0
B. pestis caviae, E. and C. 6	–	A	–	A	A	A	–	A	–	–	A	A	A	–	A	–	–	A		A
B. typhi murium	–	A	–	A	A	A	–	–	–	–	A	A	A	–	A	–	–	A		A
Hog cholera	–	–	–	A 0.8	–	A 0.4	–	–	–	–	A 0.1	A 0.4	A 0.3	–	A 0.6	–	–	–		A 0.1
B. abortus equi	–	A 0.5	–	A 0.4	A 1.5	A 0.8	–	–	–	–	A 0.2	A 0.5	A 0.8	–	A 0.6	–	–	A 0.9		A 0.5

A, Acid production.

–, no acid produced.

D, delayed fermentation, no change in first 72 hours.

* Figures under A indicate the production of gas in volumes or tenths of a volume of the amount of medium used.

TABLE II

Direct agglutination tests with muriotitis immune serum and antigens of paratyphoids from other species

ANTIGEN PREPARED FROM CULTURE OF	SERUM OF RABBIT 86 IMMUNIZED TO MURIOTITIS STRAIN NUMBER 4. TITER 32,500								
	20	100	500	1,000	2,000	4,000	8,000	16,000	32,000
Muriotitis, strains 1, 2, 3, 4, 5, and 6	++	++	++	++	++	++	++	++	+
Paratyphoid B, human	+	+	+	±	–	–	–	–	–
Enteritidis	+	+	±	–	–	–	–	–	–
Pestis caviae, 146	+	+	+	±	–	–	–	–	–
Pestis caviae, E. and C. 6	+	+	+	+	±	–	–	–	–
Typhi murium	+	+	+	±	–	–	–	–	–
Hog cholera	+	±	–	–	–	–	–	–	–
Abortus equi	+	+	+	±	–	–	–	–	–

++ indicates complete agglutination with fluffy sediment and clear supernatant fluid.

+ marked agglutination with small amount of compact sediment and turbid fluid.

± slight agglutination; small flocculi observed with hand-lens, no sediment.

– no agglutination.

through animals. Sub-inoculation from fermented rhamnose broth cultures does not increase the avidity for this sugar. In the fermentation of this carbohydrate the organism appears to differ from all the other paratyphoids studied.

The hydrogen-ion concentration in dextrose broth, after 5 days' incubation, is pH 5.1, while xylose and galactose give a concentration of pH 5.3. Rhamnose broth drops only to pH 6.0 in the same length of time. After 10 days' incubation, however, it also reaches pH 5.3.

PATHOGENICITY FOR ANIMALS

Subcutaneous injections of from 0.1 to 1.0 cc. of a 24-hour bouillon culture in guinea-pigs and rabbits produce an intense local reaction with abscess formation. The animals invariably show loss of weight, pyrexia and are apparently quite ill, but eventually recover. In rats, 0.2 to 0.4 cc. subcutaneously is almost always fatal in from 10 to 18 days, the bacilli being recovered from the heart's blood and spleen.

Intraperitoneally, 0.002 cc. will kill white mice, 0.1 cc. white rats and guinea-pigs, while 0.2 cc. is the lethal dose for rabbits. The animals show a serous or fibrinous peritonitis depending upon the time of death. In late cases the spleen is enlarged and degeneration is present in the liver and kidneys.

SEROLOGICAL STUDIES

Macroscopic agglutination tests were made with different stock immune sera. The antigen used was a salt solution suspension of the bacilli grown on agar. With typhoid, paratyphoid A and B, hog cholera and enteritidis there was no agglutination above a dilution of 150.

Agglutinations were also carried out with the serum of rabbit 86 immunized to muriotitis 4 and antigens prepared from the other organisms studied. The results are summarized in Table II.

The immune serum of muriotitis agglutinates the other paratyphoids in a dilution of 1000, or below. Such agglutination was never complete.

TABLE III

Agglutination absorption experiments with muriotitis immune serum

ABSORBED WITH	SERUM OF RABBIT 86 IMMUNE TO MURIOTITIS ORIGINAL TITER 32,500								ABSORPTION CONTROL ANTIGEN OF ABSORBING STRAIN WITH LOWEST DILUTION OF SERUM
	20	100	500	1,000	2,000	4,000	8,000	16,000	
Nothing	++	++	++	++	++	++	++	++	−
Paratyphoid B, human	++	++	++	++	++	++	+	+	−
Enteritidis	++	++	++	++	++	++	+	+	−
Pestis caviae, 146	++	++	++	++	++	++	+	±	−
Pestis caviae, E. and C. 6	++	++	++	++	++	++	+	±	−
Typhi murium	++	++	++	++	++	++	+	+	−
Hog cholera	++	++	++	++	++	++	++	+	−
Abortus equi	++	++	++	++	++	++	++	+	−

The sediment was only slight and very compact, of the character observed in controls after long standing, while the supernatant fluid was also turbid. This group agglutination was so slight that absorption experiments seemed unnecessary to identify the organism as a distinct strain.

In spite of the very definite results of direct agglutination, numerous absorption experiments were done. They all show that none of the paratyphoids, available for this study, will absorb the agglutinins from muriotitis immune serum, and, conversely, that the latter organism will not remove from the sera of animals immunized to other paratyphoids the agglutinins for the immunizing strains. Table III summarizes the agglutinin absorption experiments with muriotitis immune serum.

DISCUSSION

Since Loeffler, in 1889, isolated a typhoid-like organism, Bacillus typhi murium, from white mice during an epidemic in his colony, many authors have reported similar bacilli associated with animal or human disease. The year before Gartner (6) had reported Bacillus enteritidis which he recovered from the flesh of a cow supposed to have been suffering from intestinal disease, and from the spleen of a man who died as a result of eating meat prepared from it. Smith (7), in 1891, described Bacillus suipestifer, isolated in 1885 by Salmon and Smith (8), then regarded as the cause of hog cholera, and in 1894 Smith and Moore (9) remarked its similarity to typhi murium and enteritidis. Nocard (10) isolated another which he called Bacillus psittacosis in connection with epidemic pneumonia in man traceable to infection from parrots. During the next four decades there were many reports of bacilli similar to these associated with human or animal disease. Those isolated from man have been reported by Van Ermengem (11), Poels and Dhont (12), Roman (13), Schottmüller (14), Gwyn (15), Cushing (16), Longcope (17), Hirschfeld (18), Winslow (19), Salthe and Krumwiede (2), and many others. From a variety of animals paratyphoid bacilli have also been isolated by the following: Smith (9, 20, 21), de Nobele (22), Petrie and O'Brien (23), Meyer and Boerner (24), Bainbridge (25), Krumwiede (4), Tenbroeck (26, 27), and others.

Little was known about the relation of these organisms to one another until Schottmüller (28), in 1900, showed that they could be separated into two well-defined groups by cultural differences. These he called Paratyphoid Alpha and Beta. The B group included those of animal origin. The classification of the members of the latter remained difficult and confusing due to similar cultural characteristics and the presence of marked cross agglutination reactions. The recent use of the rarer carbohydrates and the higher alcohols showed cultural differences, and the introduction of agglutinin absorption experiments suggests that many of these bacilli are identical in spite of different host origins.

By these two means, particularly the latter, the paratyphoid B bacilli have been separated into several well defined subgroups such as (*a*) Bacillus paratyphoid B, of human origin, or Bacillus schottmülleri, as suggested by Winslow (30); (*b*) Bacillus enteritidis,

also from man but recently found in swine suffering from hog cholera (26); (*c*) a large group of animal paratyphoids from a variety of species, for a type strain of which Smith has suggested the name Bacillus pestis caviae; (*d*) the bacillus of hog cholera or B. suipestifer; (*e*) B. abortus equi.

Because of the ability of these bacteria to produce human disease their classification and relation to animals become an important consideration. Recently, Salthe and Krumwiede (2) have reported an epidemic of food infection caused by Bacillus pestis caviae. They also regarded as identical an organism isolated by Winslow (19) during an epidemic caused by infected mayonnaise dressing. Hirschfeld (18) described an epidemic of paratyphoid fever due to an organism which Tenbroeck (29) later studied and placed in the hog cholera group. The last author has also reported the finding of bacilli of the animal paratyphoid group and also Bacillus enteritidis in swine suffering from hog cholera. Many such animals undoubtedly find their way into our food supply. Accidental infection with a paratyphoid of calf origin (B. aertrycke) has been reported by an investigator working with Salmonellas (31). With the more complete serological means of identification of these organisms now at our command, it seems that the commonest aetiological agents of food infection in man are the members of the several subgroups of paratyphoid bacilli so universally distributed among various species of animals.

Krumwiede (4) has shown that host origin of a culture is not necessarily an index of biological individuality and concludes that the classification of strains according to the animal host from which they are derived is not justified. Tenbroeck (27) suggests that every attempt be made to place newly isolated paratyphoid organisms in one or the other of these well recognized groups.

The question arises concerning the classification of muriotitis. Culturally it is very similar to other paratyphoids but differs in its low avidity for rhamnose and the absence or only slight production of gas in all of the carbohydrates except xylose.

By direct agglutination it seems unlike any of the others. Serum of animals immune to them does not agglutinate it to anywhere near the titer limit, and such as does occur in the lower dilutions is never complete. Conversely, its immune serum will only agglutinate the other paratyphoids incompletely in low dilutions. Agglutinin absorptions leave no doubt concerning this apparent individuality.

As muriotitis does not fall into any of the existing groups, and if further study does not prove it to be identical with some organism already reported, but whose agglutinin absorption relation to the other paratyphoid bacilli has not yet been fully studied, in the light of our present knowledge of these bacteria it must be placed by itself in a new subgroup.

SUMMARY

1. A bacillus of the paratyphoid group, apparently unencountered by previous investigators, is reported.

2. Culturally it is similar to the other paratyphoids but can be differentiated from them by its low avidity for

rhamnose, failing to ferment this sugar in 24 hours, and the absence, or only slight production, of gas in all carbohydrates except xylose.

3. Agglutination tests leave no doubt concerning its individuality and agglutinin absorptions sharply separate it from the other paratyphoid types.

4. The organism is pathogenic for mice, rabbits, guinea-pigs, but especially for rats.

5. For the bacillus here described I propose the name Salmonella muriotitis.

I am grateful to Dr. W. G. MacCallum and Dr. J. H. Brown for suggestions and assistance in the preparation of this paper.

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The Effect of Carbon Dioxide on Cells in Tissue Cultures

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STUDIES have been carried out to note the effect of carbon dioxide on the respiratory and circulatory systems, and upon lower forms of life, both plant and animal, but as yet little has been done with tissue grown *in vitro*. It is for this reason that the following observations were made on connective-tissue cells grown outside the organism, inasmuch as observations can be made on these living cells with but little injury except that produced by the carbon dioxide itself.

METHODS

All cultures were made from subcutaneous connective tissue of the neck of chick embryos, of 6 to 8 days' incubation, grown in Locke-Lewis solution—85 cc. Locke solution (NaCl 0.9, KCl 0.042, $CaCl_2$ 0.025, $NaHCO_3$ 0.02) plus 15 cc. chicken bouillon, plus 0.5 per cent dextrose—in a warm box at 39°C. and observed at intervals of 24 hours to 10 days after explanting.

The apparatus first used consisted of a carbon dioxide generator, two wash bottles, one containing distilled water and the other a few drops of silver nitrate solution in distilled water, and a delivery tube with a fine capillary point, which could be inserted through the vaseline ring of the culture and into the chamber, permitting the carbon dioxide to strike the growth in the drop of medium directly above. The carbon dioxide escaped from the culture around the opening in the ring made by inserting the delivery tube. As this apparatus demanded constant attention, it was discarded and the gas obtained from a cylinder such as is used for freezing tissues for sectioning. There was no difference in the effects produced. With the ordinary cylinder containing the gas under pressure, it was possible to regulate a rather constant flow, varying, as desired, from a few to several hundred cubic centimeters per minute. The carbon dioxide was led through a series of three wash bottles containing distilled water, the last two of which were kept at a temperature of 36° to 40°C. in order that the carbon dioxide would be at the temperature of the living tissue, and saturated with water vapor at that point to prevent absorption of moisture from the culture, thereby disturbing the concentration

[1] I wish to express my indebtedness to Dr. W. H. Lewis and Mrs. M. R. Lewis, in whose laboratory the work was carried out, for their kind assistance and suggestions.

of the salts within it. In order to be sure that drying would not take place, a special study chamber was made and several drops of Locke-Lewis solution placed in it, so that a ring of fluid extended below the growth in such a manner that light could pass unobstructed from the substage condenser through the growth to the objective above. This chamber consisted of a brass ring about 1.5 cm. in diameter and about 4 mm. high, sealed with paraffin to a plain glass slide, and with two openings, one permitting the entrance, the other the escape of carbon dioxide.

Before cultures were exposed to carbon dioxide, the apparatus was flushed with the gas and the cultures on the coverslip were inverted and sealed with vaseline to the chamber while the carbon dioxide was passing through. Studies were made on all cells before they were exposed to the gas, during the desired interval of exposure, and afterwards, the coverslip having been replaced on the original slide. In this way changes could readily be detected, except for the few seconds necessary to transfer the growth from the slide to the study chamber and refocus the objective on the cell under observation.

Some cultures were washed with fresh Locke-Lewis solution after exposure to carbon dioxide, others were repeatedly exposed to the gas, while still others were stained with neutral red and observed before and after exposure to carbon dioxide.

All hydrogen-ion determinations of the cultures and of the Locke-Lewis solutions were made by the colorimetric method devised for tissue cultures by Felton (Lewis and Felton, 1922).

CHANGES OBSERVED

The observations can be divided into those made on the cell in the resting stage and those made during division. They differed somewhat and will therefore be considered separately.

Resting stage

The fibroblast in tissue cultures is usually a somewhat flat, elongated cell with fine protoplasmic processes, attached to similar cells, so that the growth spreads out loosely with no interchange of protoplasm between the cells. The round or oval nucleus is slightly less transparent than the cytoplasm, which contains granules and vacuoles to a greater or less degree, depending upon the age of the growth (Lewis and Lewis, 1915), and fine rod-like mitochondria, which can frequently be seen in motion, bending here and there and almost constantly altering their shape. Towards the edge of the cells the number of granules and mitochondria becomes less, and at the periphery they are frequently absent. Here the cytoplasm is spread out into such a thin, transparent sheath that at times the actual outline of the cell can scarcely be made out. Within the nucleus one finds one or more nucleoli.

After the application of carbon dioxide there was little change in the shape of the cell. Sometimes there was a slight retraction of the cytoplasm and the protoplasmic fibrils linking the cells became elongated and thinned, but this was not constant and was probably due to changes brought about by individual irritability of the cultures. The cytoplasm did not undergo much change, except that, when

large amounts of gas were used, it appeared to be rendered less transparent. This was accompanied by a similar change in the nucleus. The nucleoplasm became slightly granular, resembling ground glass. The nuclear membrane assumed a sharper outline and the nucleoli were more easily seen.

With smaller amounts of carbon dioxide, the nucleus maintained the same shape as before treatment, the nucleoplasm became more transparent, the faint, almost imperceptible, nuclear membrane was seen with greater difficulty, and at times the nucleus could not be differentiated from the cytoplasm surrounding it. The nucleolus, too, coincident with the increasing transparency of the nucleus, became more and more difficult to recognize and seemed to merge, in appearance at least, with the nucleoplasm. The mitochondria did not change in shape, but movement became less pronounced and even ceased.

In cultures stained with neutral red before exposure to carbon dioxide, the changes were more constant. The main differences between them and the unstained cultures were in the appearance of the granules and in the nucleus and nucleoli. As is well known, the color shown by neutral red solutions depends to a certain extent upon the hydrogen-ion concentration of the medium in which it is dissolved. In alkaline solutions the neutral red is quite yellow or buff, with barely a suggestion of red. As the solution approaches the acid side with the higher hydrogen-ion concentration, the color becomes more and more red, until on the acid side it is a purplish rose with no suggestion of yellow. Hence, since both the granules and vacuoles of the cells in cultures become stained with neutral red, it was thought possible to note what changes in hydrogen-ion concentration, if any, took place in these bodies under the influence of carbon dioxide. Accordingly, a number of vitally stained preparations were examined before, during, and after exposure to the gas. The results were not marked in cells grown in Locke-Lewis solution with a pH 6.6 to 7.0. At this pH the granules were red with a faint tinge of yellow, and the change in color, if any, towards a purplish rose was slight. In cells in a culture medium of pH 7.6, the granules were yellowish in color. Carbon dioxide was applied and the granules became more rose-colored. In this growth, however, the range of pH was great enough to be distinctly recognized. The removal of the culture from the carbon dioxide chamber resulted in a change of the rose hue to a more orange-red color. Carbon dioxide retarded the movement of the granules and in some instances complete cessation of movement resulted. This returned somewhat after the gas was withdrawn.

The nucleus in the vitally stained cell always became more granular in appearance and much more distinct after being subjected to carbon dioxide. The nuclear membrane, too, became more readily recognizable and the nucleoli quite dense and easily seen. This was in distinct contrast to what usually occurred in non-stained preparations, for in the latter the nucleus became more transparent and the nucleoli almost invisible. This

change was most striking when several cultures of the same age and tissue were treated with the gas, some before, others after neutral red was applied; in the former greater transparency occurred, while in the latter visibility was enhanced.

Dividing cells

The effect of carbon dioxide was most marked in cells which were undergoing division. It is reasonable to suppose that cellular activities are considerably disturbed at this stage, so that changes in the environment may produce marked effects. This was the case when carbon dioxide was applied, and no predictions could ever be made concerning the detailed behavior of the dividing cells at this time.

In general, the following retardation of division took place in the treated cultures. In prophase there was a general arrest of cellular activity and it was usually an hour or two before division was completed, although in the controls division progressed to the same extent in ten to fifteen minutes. When the cells had reached metaphase or anaphase, division continued but at a rate slower than normal. In telophase, there was little or no retardation.

In some preparations it was noted that the entire equatorial plate moved slowly from one pole to the other pole without a division of the chromosomes. In other preparations, the dividing cell gradually rounded and violent cytoplasmic movements took place in a circular manner. These movements continued for several hours, after which the cell resumed the appearance of a fibroblast. In no instances were binucleate cells seen to form.

In a few cultures made from a four-day embryo, heart muscle was found beating. Carbon dioxide definitely retarded the rate and degree of contraction, in some preparations bringing about complete cessation. The withdrawal of the gas, however, was attended by a return of the heart beat; indeed, in a few instances, with an increased rate and degree of contraction. W. H. Lewis (1924) found that an acceleration occurred when a preparation of heart muscle was placed in a covered dish, and suggested that this may have been due to a slight accumulation of carbon dioxide. In our experiments the amount of carbon dioxide was much greater than could accumulate from cellular activity in so short an interval, and it is quite probable that slight amounts have a stimulating action, while larger amounts have a paralyzing effect on living tissue.

DISCUSSION

The behavior of connective-tissue cells *in vitro* towards carbon dioxide is quite similar to that observed in other tissues and organisms. That it could decrease both the rate and the size of the heart beat was shown by Jerusalem and Starling (1910) and by Ketcham, King, and Hooker (1912). Jacobs (1912), studying protozoa, noted that carbon dioxide acted as a depressant, that movements gradually ceased and death resulted. In Paramecium caudatum, he noted that the nuclei began to stand out sharply and coagulation of the protoplasm occurred, a suggestion that the

effect was due to a change in the hydrogen-ion concentration, inasmuch as this appearance is similar to that which results when cells are treated with acids (M. R. Lewis, 1923). Later, however, Jacobs (1922) noticed that there was a preliminary period of liquefaction which was followed by increasing reversible solidification, and finally by an irreversible coagulation. The period of liquefaction, an alkaline reaction, is probably what occurred when, in our preparations, the nucleus and nucleoli became more transparent and could not be differentiated from the cytoplasm. It was only when the carbon dioxide was applied for a long time or in large amounts that coagulation occurred, except in the cells which were stained with neutral red, in which slight amounts produced beginning coagulation.

Why preparations which were stained with neutral red should show a reaction different from that in unstained cultures cannot be answered, but it is suggested that, since neutral red is reducing, it may produce other disturbance in cellular physiology besides merely that of staining granules and vacuoles.

Living cells are freely permeable to carbon dioxide, oxygen, and ammonia, and it is reasonable to suppose that some changes should follow from an alteration of the hydrogen-ion concentration, but Jacobs (1920a), from studies on tadpoles with numerous solutions of the same pH as carbon dioxide, concluded that the carbon dioxide itself is an important factor.

Smith and Clowes (1924) and Smith (1925) have shown that carbon dioxide exerted a marked inhibitory influence on the velocity of division of the normally fertilized eggs of marine animals, which could not be attributed to the hydrogen ions present in the solution.

Carbon dioxide can change the hydrogen-ion concentration of the medium rapidly, as illustrated when a drop of methyl red (orthocarboxybenzene azodimethylaniline, pH 4.4 to 5.6) was added to a culture and sealed over the carbon dioxide chamber. The gas almost immediately changed the yellow indicator to the rose color corresponding to pH 4.8. This color remained as long as the gas was passing through the chamber, but the medium gradually returned to its original hue when the gas was withdrawn.

With other acids (M. R. Lewis, 1923) coagulation would have taken place at pH 4.6 to 4.4, but in my experiments this seldom occurred except with the application of large amounts of the gas. In studies on Symphytum peregrinum, Jacobs (1920b) showed that an intracellular change in hydrogen-ion concentration could occur from the use of neutral and even alkaline solutions containing carbon dioxide.

Pearce (1925), using half molar solutions of $NaHCO_3$, modified with carbon dioxide to pH 7.4, on human blood cells stained with neutral red, noted that there was a marked change in the vitally stained bodies towards the acid side.

In my series of experiments I was unable to detect these great changes in the color of the granules and vacuoles when the cells were stained with neutral red, and frequently it was felt that the change in color, if any, was very slight That carbon dioxide

could produce a change in the color of the granules was noted only in cultures that had been grown in Locke-Lewis solution at pH 7.6. The withdrawal of the gas was not attended by a distinct reversal in the color to the neutral point. It is probable that factors other than carbon dioxide played a rôle in producing the marked color change in the granules and vacuoles, as Pearce's solutions were not physiologically balanced and capable of sustaining growth and division. In all probability, then, the results obtained with cultures in Locke-Lewis solution are more representative of conditions as they exist in living tissue, and the changes observed may be ascribed directly to the influence of carbon dioxide.

But although it would seem that carbon dioxide brings about an intracellular increase in hydrogen-ion concentration, it does not follow that changes in the color of the neutral red and other dyes within the cell are an indication of the reaction of the cell (Mathews, 1921).

The presence of acid in the media of tissue cultures reduces the stain in granules and vacuoles (Lewis and Lewis, 1924). Since the repeated application of carbon dioxide in this series of experiments resulted in a gradual fading of the color of the granules, with its partial return on the withdrawal of the gas, there is a suggestion that it may have behaved as an acid.

In dividing cells, carbon dioxide shows extraordinary effects in retarding the rate of division, and the great delay in the division of the cytoplasm suggests that, under certain pathological conditions, the nucleus may divide but the cytoplasm remain intact, resulting in the formation of two or more nuclei within one cell. That giant cells form in cultures of blood after incubation for eight to ten days (M. R. Lewis, 1925) suggests the possibility that the carbon dioxide, which necessarily must have accumulated in the sealed chamber during that interval, may have played a rôle.

Hooker (1912), Harvey (1922), and others have shown that the paralyzing effect of carbon dioxide upon the tissues of the body is not due to oxygen deprivation, for the effect is present in an atmosphere of oxygen when the carbon dioxide content is higher than usual.

A few observations were made with oxygen and, although no conclusions can be drawn from so few studies, there was an indication that it produced little effect. It may be that granules and vacuoles form because of a lack of oxygen, as was suggested by Prigosen (1921), but it is not due to an increase in the carbon dioxide tension, since the cultures examined several days after treatment with carbon dioxide has no more granules or vacuoles than the controls.

Lillie (1909) thinks that carbon dioxide is the reaction product whose rate of removal from the cell determines the velocity of the chemical processes concerned in stimulation and contraction. The retardation which results in an excess of carbon dioxide in these studies is interesting from this viewpoint.

The effect on mitochondria is not a specific one, since pyrogallic acid (Bauer, 1923), other acids (M. R. Lewis, 1923), and possibly other substances can produce similar changes.

Many of the cultures were dead at the end of 24 hours, although in several instances the carbon dioxide seemed to have no effect on the viability of the cells, for the cultures remained in good condition for days after treatment and in no way differed from the controls. The difference in behavior cannot be explained entirely by the amount of carbon dioxide, although, in general, increased volumes and duration of exposure tended to bring about death more quickly. Occasionally, while cells were being treated, a few here and there in the cultures showed lethal changes, the majority being unaffected.

CONCLUSIONS

1. Carbon dioxide had a paralyzing effect on the connective-tissue cells *in vitro*, inhibiting the movement of the mitochondria and granules and retarding division greatly in prophase, to a lesser degree in metaphase and anaphase, and only slightly in telophase.

2. In unstained preparations the cytoplasm, nucleus, and nucleoli became more transparent with carbon dioxide and suggested increased fluidity of the protoplasm.

3. In preparations stained with neutral red there occurred a slight change in color of the neutral red granules and vacuoles and also some coagulation of the nucleus which seemed to indicate that the carbon dioxide behaved as an acid.

4. Carbon dioxide was relatively non-toxic in the amounts used, but could produce death after prolonged use or with increased amounts.

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The Johns Hopkins Medical Research Club

Abstracts of Papers Presented

Stock-Plasma for Tissue Cultures. By E. C. Craciun. *From the Department of Pathology. Presented October 24, 1925.*

The preparation of blood-plasma for tissue cultures can be very much simplified by the use of heparin which is one of the physiological anticlotting substances present in mammalian blood (Howell and Holt, 1918, Howell, 1925). The heparin used in this work was furnished by the courtesy of the manufacturers, Hynson, Westcott and Dunning, Baltimore.

Such a heparin-plasma may be kept in glass ampules, ready for use and without change of its clotting power, for as long as 5 to 7 months. In order to obtain plasma, 15 to 20 cc. of blood are quickly drawn (by heart or aorta puncture) into a syringe which contains 1 cc. of a 1:1000 heparin solution. If there has been no contamination with tissue juice during the bleeding and if the heparinated blood is cooled for 10 to 15 minutes in an ice-bath immediately after withdrawing it from the animal, such small amounts of heparin are quite sufficient to prevent clotting for a number of months. The clear plasma obtained by centrifugalizing is transferred with a pipette to 1 cc. ampules and sealed.

No paraffine, vaseline or olive oil is necessary for preparing the glassware, which should be made of non-soluble glass.

The heparin is made up in solution in 0.85 per cent saline (pH 7.0), put in 1 cc. ampules and sterilized in the autoclave.

In order to avoid the use of an accidentally infected plasma, one ampule of each new sample is incubated for 72 hours, after which smears and cultures, or few a tissue cultures, are made.

Heparin-plasma is suitable for tissue culture work because: (1) It may be kept unclotted for a long time. In this way plasma of uniform composition may be used in several series of cultures. (2) It clots firmly in a short time after coming into contact with the explant. (3) Plasma with 0.005 to 0.04 per cent heparin gives as good a growth as ice-plasma, both for connective and for epithelial tissues.

Vaccinia-Virus in Tissue Cultures. By E. C. Craciun and E. H. Oppenheimer.

Although many attempts to grow the vaccine virus by ordinary bacteriological methods have failed, this virus can be cultivated in tissue cultures as shown by Steinhardt, Israeli and Lambert (1913), and Parker (1924). Knowing, then, that vaccinia virus will grow combined with the living cell, we attempted to grow only that part of it which is suspected of being its potent factor. This consists of the "granules" obtained by W. G. MacCallum and E. H. Oppenheimer (1924) by their method of "differential centrifugalizing" and seen easily by means of dark field illumination.

In these experiments the granules were separated from all other material in the virus by the above method and were further purified by washing in Locke's solution and again precipitating with centrifugation. After making certain that no contaminating substance remained, they were made up to their original concentration in Locke solution used for the tissue cultures.

Tissue cultures of rabbit embryonal cornea were put in homologous ice-plasma or in heparin-plasma (E. C. Craciun, 1925) and pure epithelial growth was obtained practically in every culture. A certain amount of the purified virus was added to these cultures. The pure epithelial cultures were carried further; they were washed and changed to a new drop of medium every 3 to 7 days. A new piece of tissue was

added each time. Every 8 days the presence of the virus was tested by inoculation on the rabbit cornea.

It is known that vaccinia virus when incubated alone at 37°C. will be greatly weakened after 7 days and dead in three weeks (Steinhardt, Israeli and Lambert). In one of our series, the virus not only was alive after 24 days, as proved by a positive "take," but it had increased either in quantity or in virulence in the meanwhile, because earlier inoculations, after 8 and 15 days, with cultures from the same series gave no "take" at all.

Further experiments on the cultivation of the granules are under way.

Books Received

Some Fundamental Considerations in the Treatment of Empyema Thoracis. By EVARTS A. GRAHAM, A.B., M.D. Illustrated. 1925. 8°. 110 pages. The C. V. Mosby Company, St. Louis. $2.50.

Personal and Community Health. By CLAIR ELSMERE TURNER. Illustrated. 1925. 8°. 426 pages. The C. V. Mosby Company, St. Louis. $2.50.

The Normal Diet. A simple statement of the fundamental principles of diet for the mutual use of physicians and patients. By W. D. SANSUM, M.S., M.D. Illustrated. 1925. 12°. 72 pages. The C. V. Mosby Company, St. Louis. $1.50.

Old and New Viewpoints in Psychology. By KNIGHT DUNLAP. 1925. 12°. 166 pages. The C. V. Mosby Company, St. Louis. $1.50.

Methods in Surgery. Used in the surgical divisions of Barnes Hospital, St. Louis Children's Hospital, and Washington University Dispensary, including outlines for case history-taking, preoperative and postoperative care of patients, routines, diets, etc. By GLOVER H. COPHER, M.D. 1925. 12°. 232 pages. The C. V. Mosby Company, St. Louis. $3.00.

Symptoms of Visceral Disease. A study of the vegetative nervous system in its relationship to clinical medicine. By FRANCIS MARION POTTENGER, A.M., M.D., LL.D., F.A.C.P. Third edition. With eighty-six text illustrations and ten color plates. 1925. 8°. 394 pages. The C. V. Mosby Company, St. Louis. $6.50.

Textbook of Orthopedic Surgery. For Students of Medicine. By JAMES WARREN SEVER, M.D. 1925. 8°. 353 pages. The Macmillan Company, New York. $4.50.

Allergy, Asthma, Hay Fever, Urticaria and Allied Manifestations of Reaction. By WILLIAM W. DUKE, Ph.B., M.D. With seventy-five illustrations. 1925. 8°. 339 pages. The C. V. Mosby Company, St. Louis. $5.50.

Bone Sarcoma. An Interpretation of the Nomenclature used by the Committee on the Registry of Bone Sarcoma of the American College of Surgeons. By E. A. CODMAN, M.D. With twenty-four illustrations. 1924. 12°. 93 pages. Paul B. Hoeber, New York. $2.00.

Eye, Ear, Nose and Throat Manual for Nurses. Bt ROY H. PARKINSON, M.D. Illustrated. 1925. 12°. 207 pages. The C. V. Mosby Company, St. Louis. $2.25.

Ocular Therapeutics. A Manual for the Student and the Practitioner. By Doctor Ernst Franke. Translated by CLARENCE LOEB, A.M., M.D. 1925. 8°. 183 pages. The C. V. Mosby Company, St. Louis. $3.50.

Physiological and Clinical Chemistry. By WILLIAM A. PEARSON, Ph.C., Ph.D., M.D., and JOSEPH S. HEPBURN, A.M., B.S. in Chem., M.S., Ph.D. Illustrated. 1925. 8°. 306 pages. Lea & Febiger, Philadelphia and New York. $4.00.

The Healing Gods of Ancient Civilizations. By WALTER ADDISON JAYNE, M.D. 1925. 8°. 569 pages. Yale University Press, New Haven. $5.00.

The Surgery of Pulmonary Tuberculosis. By JOHN ALEXANDER, B.S., M.A., M.D. With introductions by Hugh Cabot, M.D., C.N.G., LL.D., F.A.C.S., and Edward R. Baldwin, M.A., M.D. Illustrated with 53 engravings and 13 plates. 1925, 8°. 356 pages. Lea & Febiger, Philadelphia and New York. $4.50.

Gynecologic Urology. By LYNN LYLE FULKERSON, A.B., M.D., F.A.C.S. With 166 illustrations, including 86 original and 14 color plates. 1925. 8°. 247 pages. P. Blakiston's Son & Co., Philadelphia. $6.00.

Feeding and the Nutritional Disorders in Infancy and Childhood. By JULIUS H. HESS, M.D. Illustrated with forty-two engravings in the text and one full-page colored plate. Fourth revised and enlarged edition. 1925. 8°. 556 pages. F. A. Davis Company, Philadelphia. $4.50.

Physiological Chemistry. A Textbook and Manual for Students. By ALBERT P. MATHEWS, Ph.D. Fourth edition. Illustrated. 1925. 8°. 1233 pages. William Wood & Company, New York. $6.50.

Diseases of the Nose, Throat and Ear. Medical and Surgical. By WILLIAM LINCOLN BALLENGER, M.D. Revised by Howard Charles Ballenger, M.D. Firth edition. Illustrated with 551 engravings and 32 plates. 1925. 8°. 1080 pages. Lea & Febiger, Philadelphia. $10.00.

An Introduction to Objective Psychopathology. By G. V. HAMILTON, M.D. With foreword by ROBERT M. YERKES, Ph.D., LL.D. 1925. 8°. 354 pages. The C. V. Mosby Company, St. Louis. $5.00.

A Textbook of Pathology. With a final section on Post-Mortem Examinations and the Methods of Preserving and Examining Diseased Tissues. By FRANCIS DELAFIELD, M.D., LL.D., and T. MITCHELL PRUDDEN, M.D., LL.D. Thirteenth edition. Revised by Francis Carter Wood, M.D. With eighteen full-page plates and eight hundred and ten illustrations in the text in black and colors. 1925. 8°. 1354 pages. William Wood & Company New York. $10.00.

An Intermediate Textbook of Physiological Chemistry with Experiments. By C. J. V. PETTIBONE, Ph.D. Third edition 1925. 8°. 404 pages. The C. V. Mosby Company, St. Louis. $3.25.

Arteriosclerosis. A Summary View. By the late RT. HON. SIR T. CLIFFORD ALLBUTT, P.C., K.C.B., M.A., M.D. 1925. 12°. 108 pages. Macmillan & Co., Ltd., London. $2.00.

Scientific Nutrition in Infancy and Early Childhood. For the Student and General Practitioner. By STAFFORD MCLEAN, M.D., and HELEN L. FALES, B.S. 1925. 8°. 404 pages. Lea & Febiger, Philadelphia. $4.75.

The Art of Medical Treatment. With Reference both to the Patient and to his Friends. By FRANCIS W. PALFREY, M.D. 1925. 8°. 463 pages. W. B. Saunders Company, Philadelphia, $4.50.

A Textbook of Obstetrics. By THOMAS WATTS EDEN, M.D., C.M Edin., F.R.C.P. Lond., F.R.C.S. Edin., Major R.A.M.C., and EARDLEY HOLLAND, M.D., B.S. Lond., F.R.C.P. Lond., F.R.C.S. Eng. Sixth edition. With 7 plates and 393 illustrations in the text. 1925. 8°. 704 pages. The Macmillan Company, New York. $6.50.

International Clinics. A Quarterly of Illustrated Clinical Lectures and Especially Prepared Original Articles. By leading members of the medical profession throughout the world. Edited by Henry W. Cattell, A.M., M.D. and Charles H. Mayo, M.D., [*et al.*] 1925. 8°. 309 pages. J. B. Lippincott Company, Philadelphia and London. $2.00.

Essays in Psychopathology. By WILLIAM A. WHITE. Nervous and Mental Disease Monograph Series No. 43. 1925. 8°. 140 pages. Nervous and Mental Disease Publishing Company, New York and Washington.

Book Reviews

Medicine, an Historical Outline. By M. G. SEELIG. 207 pp. $2.25. (*The Williams & Wilkins Company, Baltimore, 1925.*)

Far too little interest is taken, actively at least, by the majority of medical students and doctors in the alluring and enlightening subject of medical history. "It is the sign of a day-age when great men of the past are held in light esteem." This book of 207 pages presents a series of eight lectures delivered by the author in St. Louis in 1924 to the senior students of the Washington University and St. Louis University Schools of Medicine. The subject is presented very much in brief, merely as an introduction and stimulus to further interest in the history of the profession among its members during "their plastic medical school period." It aims thus to offer "culture to the 'guild man' who knows the history of his guild."

The first two lectures deal with the story of primitive and oriental medicine and that of the Graeco-Roman Era. A trifle bewildering in the bulk of superstition that it casts upon the head of the sympathetic reader who may learn for the first time the story of early Medicine and the beginnings of Empiricism. In these, and the two lectures which follow on the Middle Ages, much space is rightly given to the influence of contemporary thought on the medical theories and progress of the times. With the Sixteenth Century we are introduced to the Renaissance awakeners, particularly "the great iconoclast Vesalius"—to Servetus, to Paracelsus, "a creature of the times . . . . by nature disputatious and disrespectful of authority, coarse to the point of vulgarity and contentious to the degree of bullying,"—and to Ambroise Paré. The three closing lectures form a chronological treatise on all that has followed, in parts disappointingly brief, though of necessity so. The author has carefully and wisely linked in many instances the names of figures less prominent to the anatomical structures by which, alas, many of them are remembered today. The work is presented in a fresh and clear style, colored with many original turns of speech which make it very entertaining and readable—a highly commendable volume for the beginner in medical history.

M. N. F.

Surgical Pathology. By JOSEPH MCFARLAND, M.D., Sc.D., Professor of Pathology in the Medical Department of the University of Pennsylvania. 701 pp. $9.00. (*P. Blackiston's Son & Co., Philadelphia.*)

In "Surgical Pathology" the author succeeds admirably in summarizing the essential facts in connection with the pathological materials with which the surgical pathologist and the surgeon have to deal. The arrangement of the subject matter is somewhat unique. However, it seems quite satisfactory, when one considers the great variety of conditions which are discussed in the text. The book contains three major divisions dealing respectively with congenital conditions of surgical interest, tumors and special pathology of the various organs which primarily concern the surgeon. As was to be expected, the writer did not succeed in avoiding confusion in his attempt at classification of the materials considered. Many of the divisions and subdivisions are arbitrary and there is much overlapping.

The section on congenital conditions of surgical interest is done with more brevity than seems desirable if the reader is to be led to a clear understanding of the more complicated congenital abnormalities. One gains a sense of incompleteness by reading the many excerpts from the detailed embryological studies which are quoted. Possibly, however, we should regard this as adequate treatment in view of the necessarily limited scope of the book.

In Part II the general consideration of

tumors is excellent. However, difficulties are again met with in the matter of classification. Notwithstanding this, the arrangement of the subject matter is simple and forms a very practical and useful guide to the subject of new growths.

Part III, devoted to special pathology of the various organs which are so frequently the site of conditions requiring surgical treatment, composes about half the text. Neoplasms here occupy a minor position in view of their previous consideration in Part II. The section on the breast is written from a point of view entirely different from that of many other writers, especially in connection with the so-called "chronic cystic mastitis." The author's attempt to show that this condition is the result of "delayed involution" is admirably done, though the argument is not always convincing.

The vast majority of the four hundred and thirty-five illustrations are accredited copies from well known older publications. Many of them are excellent. However, we cannot help feeling that this phase of the work could have been made much more attractive and far more useful by the use of more recent figures, picturing the conditions as the surgeon sees them today, rather than the extremes as they occurred in the days when surgical advice was sought as a last resort. The microphotographs leave much to be desired.

The writer has striven to make his text interesting and, in so far as material and form are concerned, has succeeded. The style, however, becomes somewhat tedious in continuous reading and is not always pleasing, especially since one is frequently annoyed by errors in the text.

W. D. F.

International Clinics. Vol. I. Thirty-fifth Series. Ed. by Henry W. Cattell, A.M., M.D., and Charles H. Mayo, M.D. *(et al.)* $2.00. *(J. B. Lippincott Co., Philadelphia, 1925.)*

This volume maintains the usual excellence of the previous ones in a sensible assortment of subjects of first importance to the practising physician. At first glance the reader might think that the articles were probably an accumulation of the same material we have been having for years; but this is certainly not the case.

Two of Dr. Barker's clinics are given, one on a case of organic disease (staphylococcus sepsis), and the other, presented in the way in which he has few equals, on "The treatment of the psychoneuroses." An article by Alfred Gordon on the "Psychoneuroses in relation to General Medicine" goes very well in connection with Dr. Barker's clinic. The other papers are most practical, only one or two being a trifle fanciful. One of the satisfactory ones is that of Dorsey and Monaco on "Some of the Urological Problems most frequently encountered in daily practice." A great many sensible points are also emphasized by J. Clarence Keeler in a concise lecture on "Mastoid Diseases," and the common pitfalls are brought out well.

The paper by Hubert Blanc on "Death from Tobacco," and an account of seven deaths from angina coinciding with indulgence in tobacco, attract the attention but leave one unconvinced.

A review of the Progress of Medicine for 1924 by Henry W. Cattell and James F. Coupal is given at the end.

T. O.

Diseases of Ear, Nose and Throat. By HAROLD HAYS, M.A., M.D., F.A.C.S. First edition. Cloth. $10. Pp. 961, with 55 plates and 495 engravings. *(Philadelphia, F. A. Davis Co., 1925.)*

As the author states in his introduction, the work is planned for the general practitioner and for this reason technical discussions are avoided. From this viewpoint the book gives a good general idea of the diseases of the ear, nose and throat and their treatment. The methods of exnmination are especially complete. The chapters on the ear cover about one half the book and are well worked up. The diseases of the nose and throat are covered in a more general way along with the methods of treatment and operation. The illustrations, though not all original, are good.

E. N. B.

An Introduction to Practical Bacteriology. By T. J. MACKIE and J. E. MCCARTNEY. 297 pp. $3.50. (*William Wood & Co., New York, 1925.*)

This volume is a student's guide for a course in elementary bacteriology. It is not intended as an epitome of the subject, but might be regarded as a sort of recapitulation, with brief notes, of a series of lectures given by the authors. The more important facts are stated briefly and the more extended discussions are obviously left for the lecturer. The treatment is from a medical and public health viewpoint. The material has been arranged for the purposes of the course in bacteriology given by the authors at the University of Edinburgh.

The subject matter includes the usual discussions of the nature of bacteria, their biochemical activities, methods of pure culture study, staining and disinfection. Brief descriptions of the common serological procedures are given, along with methods of animal inoculations, autopsies, etc. There is a brief section on the preparation of tissue sections.

The cultural reactions and methods of diagnosis of the common bacteria of interest to the physician and public health officer are outlined.

M. F.

Collected Papers of the Mayo Clinic and the Mayo Foundation. Vol. XVI, 1924. (*Philadelphia and London: W. B. Saunders Co. 1925. xix, 1331*).

The current volume maintains the standard set for this collection in recent years. One hundred and sixty contributors are listed and two hundred and twenty-four articles are printed either in full or in abstract. Many less important papers are given by title only. This makes an average of almost two papers a year from each contributor, surely a most impressive showing.

Practically no field of medicine or surgery has been neglected, although, of course, emphasis is laid on those branches of surgery for which the Mayo Clinic has become so famous. By far the most important papers are those dealing with the physiology of the liver by Mann, Bollman and Magath. Their studies are the result of brilliantly executed and carefully controlled experimental work and will undoubtedly prove to be of most important clinical and scientific value.

No new technical procedures appear, and the alimentary tract problems are largely of a statistical nature. The tremendous number of cases passing through the hands of this group of surgeons gives such papers a distinct value. Many articles of a general character and several important ones on syphilis also go to make up the volume.

The method of compiling the bibliography in many of these published articles is open to some criticism, if a bibliography is regarded as a means of giving to the reader original sources of information. "Quoted by" occurs somewhat too frequently. Many inaccurate quotations may easily be perpetuated in this way and the method has no value so far as ready access to the literature is concerned.

These Mayo volumes have already gained an established place in every medical library. The present one falls easily in line with its predecessors.

W. H.

Bone Sarcoma. By E. A. CODMAN, M.D. 93 pp. $2.00. (*Paul B. Hoeber, Inc., New York, 1925.*)

The work of the committee on the registry of bone sarcoma of the American College of Surgeons needs no special introduction. During the four years of its existence considerable advance has been made in the difficult task of evolving a simple yet inclusive nomenclature, based on end-results as seen in the gross and microscopic anatomy and by the roentgen ray examination. Such a clearing away of obscure and often meaningless terminology was urgently needed. Dr. Codman and his associates have developed a classification based on ten varieties of bone sarcoma, which, they feel, will suffice for the present need, while at the same time offering a basis for further study.

This small book of ninety pages is made up of a discussion and an explanation of this classification with illustrative case reports, and fully justifies the plea that surgeons and pathologists should coöperate with the committee by registration and study of their cases. It should be in the possession

of every surgeon, and should be available in every laboratory. Students will find it more than useful in the clarification of a difficult problem.

E. M. H., Jr.

An Introduction to Dermatology. By Sir Norman Walker. 8th edition. Ninety-two plates and eighty illustrations. 373 pp. $7.00. (*William Wood & Co., New York, 1925.*)

This is the eighth edition of Walker's work in twenty-five years. As originally published, it was a reproduction of his lectures to students at Edinburgh. The new editions were so revised that even this last is not so very much larger than the first, despite many additional features.

One can always learn something by reading his introductory section. Especially helpful suggestions are to be found in the chapter on treatment in general, and the preparation and application of local remedies. It is disappointing, however, to find so little space given to the most important local treatment—x-ray application.

To those of us who are advocates of vaccine therapy, it will be a pleasure to know that at last Walker has recognized its value and became a proponent. Everyone should read his chapter on ringworm to learn what a scourge it may become. It is open to question, however, whether he does not go too far when he advises to keep children with alopecia areata from school. The descriptions and treatment of the syphilodermata are somewhat disappointing.

The many colored plates are well selected, but the coloring is too vivid. Most of the photographs are poorly reproduced. As good illustrations are the most important part of a textbook on diseases of the skin, these should be changed.

E. R. S.

Physical Chemistry in Biology and Medicine. By J. F. McClendon, Ph.D., Professor of Physiologic Chemistry, University of Minnesota Medical School, and Grace Medes, Ph.D., Assistant Professor of Physiologic Chemistry, University of Minnesota Medical School. Octavo of 425 pages, illustrated. $4.50 net. (*W. B. Saunders Company, Philadelphia and London,* 1925.)

As indicated in their preface, the authors have endeavored to furnish the research worker in biology and medicine with an outline of work in the physical sciences which may serve as an aid to him in acquiring a training in these fields. The book "in no way partakes of the nature of a text on all the subjects considered." It is quite comprehensive in the subjects treated and includes discussions of atomic structure, colloids, intermolecular forces, thermochemistry in the living body, radiant energy, and the more common branches of physical chemistry. This large range of subject matter naturally precludes the possibility of anything but a mere sketch of each subject. Abundant references follow each chapter, but no discrimination seems to have been chosen in their selection.

The many applications of physical chemistry to biology and medicine will be of interest to the reader. The theoretical portions of the book, however, are poor, both in form and treatment, and, above all, are lacking in unity of presentation. The discussion of free energy, which follows Ostwald's dilution law on page 78, may be quoted as an example. The application of physical chemistry to the biological sciences has passed the stage when a mere superficial knowledge of the subject matter of physical chemistry will suffice, but requires as rigid a foundation as the purely physical sciences themselves.

A. G.

Cancer and the Public. (The Educational Aspect of the Cancer Problem.) By Charles P. Childe, former President of the British Medical Association, Chairman of the Health Committee, Borough of Portsmouth, Consulting Surgeon of the Royal Portsmouth Hospital. 267 pp. $4.25. (*E. P. Dutton & Co., New York, and Methuen & Co., London.*)

This book is a delight to read. The art of the publisher is shown in the size and clearness of the type, in the lightness and the entire appearance of the book. The scholarship of the author stands out on every page, in his good English and in his knowledge of all that has been written on

the educational aspects of the Cancer Problem. There are no fads or fancies; he has no personal cause or cure for cancer, but describes and agrees with the majority opinion of those who have devoted their life studies to its cause, prevention and cure. At the present time the cause of cancer is unknown. There is no cure for late cancer. When patients delay after the first warning their chances of reaching a hopeless stage increase rapidly, while the probabilities of a cure, if the condition is still operable, grows steadily less. When the public can be given the correct information in regard to cancer, and when they seek advice the moment they are warned, cancer, in many instances, can be prevented. But when an individual seeks advice very early and the lesion is cancer, the probability of a hopeless outcome is less than 5 per cent as compared to 50 where there is delay, while the chances of a cure in the early stage are 70 per cent or more as compared with 25 or less in the later stages.

This book undoubtedly gives the best presentation of cancer as an educational problem. There are no illustrations. There are thirteen chapters with a good index, and a most excellent list of references.

In view of the publicity by the New York Times and the American Press of the excellent and most praiseworthy scientific and laboratory investigations of Gye and Barnard, which unfortunately up to the present time have not helped us in the recognition and treatment of cancer, the educational aspect of the cancer problem has been thrown into the shadow, and the clear, common-sense, and life-saving aspect, as presented in this book by Childe, has not received its justifiable publicity.

J. C. B.

Symptoms of Visceral Disease, A Study of the Vegetative Nervous System in Its Relationship to Clinical Medicine. By F. M. Pottenger. Third edition. Pp. 394. $6.50. (*St. Louis: C. V. Mosby, 1925.*)

This is a comprehensive, if somewhat uncritical, survey of the functions of the involuntary nervous system with special reference to clinical medicine. The first part includes a discussion of the anatomy, physiology and pharmacological reactions of the vegetative system and its influence on cell metabolism. The second part is devoted to a general discussion of the relation between visceral disease and the vegetative system. In the third part, the author describes in detail the innervation of the internal organs and gives an illuminating description of the vegetative reflexes which arise in disease. The reflexes are expressed peripherally as muscle spasm or atrophy, local tenderness, cutaneous hyperesthesia or referred pain. The author covers the ground adequately and gives references to the more important articles in the literature. The book is well illustrated and has an index. It should be of value to internists and surgeons who are familiar with these reflexes and their significance, but rather vague about the underlying physiology.

F. R. F.

The Principles of Public Engineering. By Earle B. Phelps, B.S., Professor of Sanitary Science, College of Physicians and Surgeons, Columbia University. Formerly Associate Professor of Chemical Biology, Massachusetts Institute of Technology. Pp. 265; 35 charts and drawings. $3.00. (*New York, The Macmillan Co.*)

In this small volume Professor Phelps has briefly but admirably discussed the most important subjects in public health engineering. The author is well known to sanitarians and has approached his subjects with the background of many years of active experience in the broad field of sanitation.

In the preface it is stated that the text contains in somewhat condensed form material which was brought together for a course in Public Health Engineering first offered at Columbia University in the summer school of 1924 and that it was prepared for two classes of advanced students; for the medical health officer who has had little or no engineering training, and for the sanitary engineer well versed in engineering but with insufficient knowledge of public health principles.

The book deals with principles rather than practice, the subjects discussed being atmospheric pollution, ventilation of buildings, municipal water supply, sewerage, the pasteurization of milk, lighting and mis-

cellaneous environmental control, the last subject treating briefly of the collection and disposal of municipal refuse, mosquito control and fly control.

It is realized that an author has to draw the line somewhere in the treatment of his subject, but it is suggested that in a second edition he might well add brief discussions of such subjects as camp sanitation, the sanitation of transportation facilities and the control of offensive odors, as well as enlarge somewhat his discussion of some of the subjects mentioned in the text.

The printing and binding are good and there is an excellent index. Another very commendable feature is the list of references and collateral reading given at the end of each chapter, but it is to be regretted that some of the cuts are poorly executed. The reviewer offers the friendly suggestion that such cuts be redrawn, as the text is far too good to be marred by poor drawings, and the further suggestion that some cuts be replaced by others more in accord with actual practice.

The book is a very readable one and both the medical health officer nnd the practicing sanitary engineer will be well repaid for a careful study of its contents.

J. H. G.

A Companion to Manuals of Practical Anatomy. By E. B. Jamieson, M.D. Second edition. 538 pp. $5.00. (*William Wood & Co., Edinburgh, London, 1925.*)

The author's aim has been "to provide, in an easily portable form, an account of naked-eye Anatomy, expressed in terms of the Bâle nomenclature." Since the book contains no illustrations, and raises no questions, it must be used merely as a summary or outline of the larger works. Its style is severely telegraphic, in places cryptic, and the subject matter is treated rather unevenly, as when the autonomic nervous system is dismissed in a brief half page.

The arrangement of the book is such that by a proper system of mnemonics one might conceivably memorize the most of gross Human Anatomy, as for a board examination or other perfunctory exercise. Thus the book may be a very good quiz compend, perhaps too good as such things go, but not good enough to justify the evident and extensive labor of its preparation. The author can hardly have offered it as a contribution to the science of Anatomy.

C. F. D.

The Nature of Enzyme Action. By Sir W. M. Bayliss, M.D., D.Sc., F.R.S., Late Professor of General Physiology, University College, London. Fifth Edition. 200 pp. $3.25. (*Longmans, Green & Co., 1925.*)

The fifth edition of this valuable contribution to the subject of enzymes, published in the Series of Monographs on Biochemistry, was carefully revised and brought up to date by Dr. Bayliss in the spring of 1923, just before he was overtaken by his fatal illness. The most important corrections and additions relate to the methods of purification of enzymes and to the problem of equilibrium in heterogeneous catalysis.

A carefully selected bibliography, comprising some 500 references to papers which have a direct bearing upon the subject, is appended.

The book deals largely with the "nature" of action of enzymes as a whole rather than with individual enzymes. These are discussed only so far as they bear upon definite enzymatic systems. This is indicated by the various headings: I. Catalysis in general. II. Enzymes as catalysts. III. Physical and chemical properties of enzymes. IV. General methods of preparation and of investigation. V. Reversibility of enzyme action. VI. The velocity of reaction and the various conditions affecting it. VII. The mode of action of enzymes. VIII. Co-enzymes and anti-enzymes. IX. Zymogens. X. Oxidation processes and certain complex systems.

The ideas of the author upon enzyme action can be best illustrated by citing from the general conclusions of the book:

"A careful study of these enzymes shows that they obey the usual laws of catalytic phenomena. Certain deviations from the behaviour of homogeneous inorganic catalysts are found to depend upon the colloidal nature of enzymes, so that the reactions take place in a heterogeneous system and the various phenomena depending upon

surface action come markedly into play as they do in the case of inorganic heterogeneous catalysts.

"So far as we know, the reactions catalysed by enzymes are reversible in nature, but since, as investigated *in vitro*, they usually take place in presence of excess of water, the equilibrium-position is very near the stage of complete hydrolysis. Owing to this reversible character of the reactions, it follows that enzymes have synthetic action. In the organism, mechanisms appear to be present which are able to diminish to a large extent the effective concentration of water, so as to favour the synthetic reaction, when required.

"Reasons are given for the belief that the "compound" of enzyme and substrate, generally regarded as the preliminary to action, is of the nature of a colloidal adsorption-compound; so that the action of enzymes in general must be regarded as exerted by their surface. By surface condensation the reacting constituents are brought into intimate contact and reaction accelerated by the powerful compressive forces of cohesion at the interface. Whether chemical combination between enzyme and substrate occurs in any stage of the process is not yet decided.

"Autocatalysis, positive and negative, plays a considerable part in the changes of activity of an enzyme during the course of its action. These changes of 'activity' are the main factors in the deviations of the form of the equation for the velocity of reaction from the simple unimolecular formula, when the reaction, as is usually the case, takes place in presence of excess of water."

S. A. W.

The Fats. By J. B. Leathes, Professor of Physiology in the University of Sheffield. and H. S. Raper, Brackenbury Professor of Physiology in the University of Manchester. Second edition. $4.50. (*Longmans, Green & Co., 1925.*)

There are three requirements of a good monograph on a biochemical subject. In the first place, it should give a thorough and systematic presentation of the established physical, chemical, and physiological facts that have accumulated in the course of the study of the subject. Again, it should coordinate the many theories and ideas, either unestablished or partly established, of the physiological significance and relationships of the subject discussed, and should authoritatively sift and weigh them. Finally, it must circumspectly enter the field of speculation, suggesting possible relationships and outlining future methods of attack of the subject. If, besides these qualifications, a monograph can offer lucidity and distinction of style, making the book pleasant to read, it is a worthy piece of work, indeed. The monograph on "The Fats" by Leathes and Raper contains all these characteristics to an easily recognizable degree.

The book is a second edition, the first having been written in 1910, by Leathes alone. That there has been a distinct need for a new edition has been obvious for a long time to workers in biochemical fields. The earlier book, though an excellent piece of work, almost immediately became out of date, if only because the outstanding researches in the physiology of fats have been done in recent years. The authors have been well aware of this fact; and it is for this reason that the emphasis in the new book is on the physiology of fats rather than on the chemistry, seven chapters out of ten being devoted to the former phase of the subject.

The whole discussion of the physiological relationships of fats and their derivatives is marked by thoroughness and sobriety of judgment. The accumulation of an enormous bibliography, which we find here, may not in itself be a noteworthy achievement, but the presentation of the facts and ideas in these papers in a way to bring out their interrelations and importance is the mark of masters in the field.

The last chapter of the book very properly deals with "the part played by fats in the life of the cell." The disappointment in finding this chapter less pithy with material than the others is alleviated by the frank recognition of the authors that in this phase of the subjects investigators have been limited because of lack of knowledge of the physical-chemical factors, and that only with the advance of physical chemistry can

we expect to obtain a real insight into the rôle of fats in the life of the cell.

W. S. H.

Physical Chemistry for Students of Medicine. By ALEXANDER FINDLAY. $2.60. (*Longmans, Green and Co.*)

This summary of the most important sections of physical chemistry for medical students is a small book of about 200 pages, non-mathematical, and very readable. The simple kinetic viewpoint is made the basis of all the exposition, without mention of the thermodynamic principals. The scope of the book may be gathered from the titles of the twelve chapters: Gas Laws, Aqueous Milieu of the Life Processes, Diffusion and Osmotic Pressure in the Living Organism, Behavior of Electrolytes in Solution, Law of Mass Action and Chemical Equilibrium, Mass Action Applied to Solutions of Electrolytes, Hydrion, Velocity of Reaction and Catalysis, Colloidal State, Adsorption, Permeability of the Cell Membrane.

There is nothing new or striking in the presentation of the physical chemistry, it being a straightforward mosaic of the beliefs and methods of exposition that have been current in physical chemical textbooks of the past two decades. One very commendable feature of the book is that the principles are invariably illustrated and applied by means of experimental data of direct interest in medicine.

One wonders what the effect on the medical student will be when he reads that electromotive forces are due to a balance between "solution pressure" and osmotic pressure, and that furthermore the quantitative value of the electrode potential may be derived by the "application of the principles of thermodynamics." Professor Findlay has attempted to simplify the intricate mass of detail found in the subject of physical chemistry and in this book he had succeeded to the extent to which the task is at present possible. This raises the question as to whether it is humanly possible to condense the necessary facts and explanations into a book of 200 pages. In the case of a negative answer to the above question which, I believe, must be given, would it not be wiser to acquaint the medical students more thoroughly with a fewer number of essential principles?

W. A. P.

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